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Genetic analysis of the behavior of a multiple gene loss induced by a transposon in maize

Yih Ching Huang
Iowa State University

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GENETIC ANALYSIS OF THE BEHAVIOR OF A MULTIPLE GENE LOSS INDUCED BY A TRANSPOSON IN MAIZE

Iowa State University PH.D. 1984

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Genetic analysis of the behavior of a multiple gene loss induced by a transposon in maize

by

Yih Ching Huang

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1. INTRODUCTION

Transposable elements in maize have been analyzed and studied for the last forty years. These transposable elements are characterized by several features such as transposition that affects gene expression and induces chromosome rearrangements. Recent studies on the molecular basis of transposable elements in maize are beginning to provide new insights into their structure and function and information on the evolution of these elements.

Maize transposable elements can insert into any gene locus and interrupt gene function. Gene activity is restored when the element is excised from the locus resulting in variegation in the kernel or plant tissues. These patterns of variegation are dependent on the timing and frequency of occurrence of mutations at precise times in plant development.

Among several controlling element systems, only the Ac-Ds system has been known to induce chromosome rearrangements in addition to the induction of instability of gene expression. In the presence of the regulatory element Ac, the chromosome is dissociated at the Ds (the receptor) site resulting at times in the formation of breakage-fusion-bridge (BFB) cycle. Chromosome duplications, deficiencies, inversions and translocations are often observed among the progeny of Ac-Ds containing plants in this system.

In addition to the gross changes in chromosome organization, changes have been seen at points of excision of elements. Recently, from the molecular analyses of the Ac and Ds elements at the Sh and Wx

loci, changes have been seen within these loci and are in a segment of the DNA sequence or only in a relatively few nucleotide pairs.

The present study is concerned with a mutliple gene loss event. It is the first case among the maize transposable elements showing such a break outside the Ac-Ds system. The allele inducing loss events represents an autonomous En insertional mutation at the A locus on chromosome 3 of maize.

The objectives of this study are:

(1) to characterize the genetic basis of this loss event;

(2) to determine the heritability of the rate of this loss event and the states of this loss event;

(3) to investigate the effects of internal and external factors on the rate of this loss event; and

(4) to study the responses to selection on the level of this loss event.

2. LITERATURE REVIEW

2.1. General Overview of Transposable Elements in Maize

Maize controlling elements are transposable elements capable of causing unstable mutations as well as a variety of chromosomal rearrangements including deletions, duplications, inversions and translocations (McClintock, 1951, 1956a, 1956b, 1978). A controlling element can insert in or near a gene and alter its ability to express the normal phenotype; for example, c-m-1 is a mutable allele with a Ds element at or close to the C locus (McClintock, 1949) and the mutable alleles of the Sh gene such as sh-m5933, sh-m6233, sh bz-m-4 each of which have the Ds element adjacent to Sh locus (McClintock, 1952).

Excision of the element, or any other type of element-associated genetic event occurring during development, can result in a regular and characteristic pattern of gene expression in the tissue of an organism. The pattern is determined by the properties of the element and is, therefore, subject to heritable alterations that affect the element.

There are two basic types of controlling systems that have been defined. They are termed one-element system (autonomous system) and two-element system (nonautonomous system). Two-element controlling element systems include two components: a trans-acting regulatory element and a cis-dominant receptor element (Fincham and Sastry, 1974; Peterson, 1981). Regulatory elements control their own excision and transposition, alter or excise receptor elements so that the locus under control becomes functional. Receptor elements suppress gene

activity when in a cis position to the locus. They are stable in their chromosomal positions unless they are influenced by the trans-activity of specific regulatory elements which may be present elsewhere in the genome. These receptor elements causing this type of insertion mutation are not known to be capable of autonomous excision and transposition (Fincham and Sastry, 1974; Fedoroff, 1983).

In the autonomous systems, the regulatory and receptor components are integral parts of the same element, both residing at the locus under control (Fincham and Sastry, 1974). Fedoroff et al. (1983b) found that both Ac wx-m-9 and Ds wx-m-9, which is a derivative of Ac wx-m-9, are almost identical at precisely the same site within the Wx transciption unit. This indicates that the Ds (receptor element) is related to the Ac (regulatory element) as integral parts of one element.

A two-element system can originate from an autonomous element through the loss of the trans-active regulatory function; following such an event, the receptor component may continue to respond to a regulatory element elsewhere in the genome. The direct evidence for the implied sequence relationship was found between Ac and Ds in Ac wx-m-9 and its derivative Ds wx-m-9. Ds is a 0.2 kb deletion of Ac wx-m-9 at the Wx locus (Fedoroff et al., 1983b).

The receptor-regulatory element relationship is highly specific. A receptor can be activated only by certain regulatory elements. This specific interaction between receptor and regulatory elements as expressed in, for example, kernel variegation, is defined as a

controlling element system. Thus far, at least six two-element systems have been defined (Peterson, 1981). They are shown in Figure 2.1. These six systems were described as a-dt-Dt (Rhoades, 1936, 1938), Ds-Ac (McClintock, 1945, 1946, 1951), I-En(Spm) (Peterson, 1953a, 1960), r-cu-Fcu (Gonella and Peterson, 1977), r-uq-Uq (Friedmann and Peterson, 1982) and o2-m(r)-Bg (Salamini, 1981). The Mp (Modulator) was found to have the same function as Ac on Ds (Barclay and Brink, 1954). Spm (Suppressor-mutator) was identified as a member of the same system as En (Peterson, 1965a). Spf was found to be related to Fcu, but Spf acts only on the r#10 allele not on r-cu (Singh et al., 1975; Gonella and Peterson, 1978). In addition, currently one more system, bz-rcy-Cy, was identified (Schnable and Peterson, 1984). Elements of all systems cause unstable insertion mutations, but elements belonging to different systems differ from each other in their mode of action.

Two of these controlling element systems that have received the most extensive analysis will be discussed in the following sections under the different subject headings: Ac-Ds (Mp) and Spm (En-I) systems.

The Ac-Ds system is the first transposable element system investigated by McClintock (1945, 1946). The essential features of this system have been summarized by McClintock (1956a). A brief description of this system will be presented here.

Ac (Activator) is a regulatory element and can be observed when it transposes to a locus whose activity can be monitored, because it may block or modulate gene activity. Ds (dissociation) is used to

Regulator \ Receptor	I a-m(r)	I a-m-1	Ds a-Ds	Ac P-vv	dt a-dt	cu r-cu	cu r#10	ruq a-ruq	I-Bg o2-m(r)	rcy bz-rcy
En	+	+	-	-	-	-	-	-	-	-
Spm	+	+	-	-	-	-	-	-	-	-
Ac	-	-	+	+	-	-	-	-	-	-
Mp	-	-	+	+	-	-	-	-	-	-
Dt	-	-	-	-	+	-	-	-	-	-
Fcu	-	-	-	-	-	+	+	-	-	-
Spf	-	-	-	-	-	-	+	-	-	-
Uq	-	-	-	-	-	-	-	+	-	-
Bg	-	-	-	-	-	-	-	-	+	-
Cy	-	-	-	-	-	-	-	-	-	+

Figure 2.1. Controlling element systems. "+" indicates that variegation has been found and "-" indicates that the test is negative (no variegation) or has not been definitively tested. Bracketed elements are related to each other (Peterson, 1981)

represent the receptor element in this system. The name "Dissociation" is derived from the observation that this element can provide a specific site of chromosome breakage or dissociation into two fragments (McClintock, 1946, 1947) when Ac is present. Ds can transpose in the presence of Ac and can insert into or become associated with a locus in such a way as to suppress the expression of the locus (McClintock, 1948, 1949). Chromosome breakage at Ds also requires the presence of Ac. Consequently, a mutation caused by insertion of Ds is unstable only in the presence of Ac. In the subsequent discussion, these properties will be described in detail under separate subjects.

Like the Ac-Ds system, the Spm(En) system contains regulatory and receptor elements. The regulatory element of this system was designated Suppressor-mutator (Spm) by McClintock (1954) and Enhancer (En) by Peterson (1953a). The receptor element was not named by McClintock and was termed Inhibitor (I) by Peterson (1953a). The Spm and En elements were identified as members of the same system through the activation criteria described by Peterson (1965a). Like other controlling element systems, this system also causes unstable mutations by the insertion-excision events at a locus. Two functions of the Spm(En) are responsible for the expression of several events at Spm(En)-controlled loci. The suppressor (s) function is responsible for inhibiting gene expression, whereas the function responsible for the reversion of gene activity is the mutator (m) function (McClintock, 1954). The s and m functions are genetically distinguishable but co-transpose (McClintock, 1955). The m function is required for both somatic and germinal instability

(McClintock, 1957, 1961, 1965b). Thus the two elements of the Spm (En) system exhibit two distinct types of interaction shown in Figure 2.2. These interactions between two elements and the action of Spm (En) will be discussed in subsequent sections.

2.2. Chromosome Rearrangements

2.2.1. General mechanisms of chromosome rearrangement

In the early studies, McClintock (1941) described the breakage-fusion-bridge (BFB) cycle in maize. The diagrams of Figure 2.3 illustrate the origin of newly broken ends of a chromosome at the meiotic anaphase and its subsequent behavior. Two types of BFB cycle were demonstrated by McClintock (1951). One is the chromatid type of BFB in which a chromosome with a newly broken end entering a telophase nucleus in the gametophytic or endosperm tissue will give rise in the next anaphase to a chromatid bridge configuration. The bridge is produced because fusion occurs between sister chromatids at the position of previous anaphase breakage. This sequence of anaphase breaks and sister-chromatid fusions will continue in successive mitosis. This cycle of chromatid type BFB is illustrated in A of Figure 2.3. However, this cycle usually does not occur in the sporophytic tissues. The broken end entering a telophase nucleus heals and its subsequent behavior resembles that of a normal, unbroken end of a chromosome.

Another type of BFB is the chromosome type of BFB cycle in which a chromosome with a newly broken end is introduced into the zygote by each gamete nucleus, and the broken ends of the two chromosomes are capable of fusion. This establishes a dicentric chromosome. In the telophase

Figure 2.2. A diagram showing the interaction between regulatory and receptor elements in Spm(En) system (modified from Fedoroff, 1983)

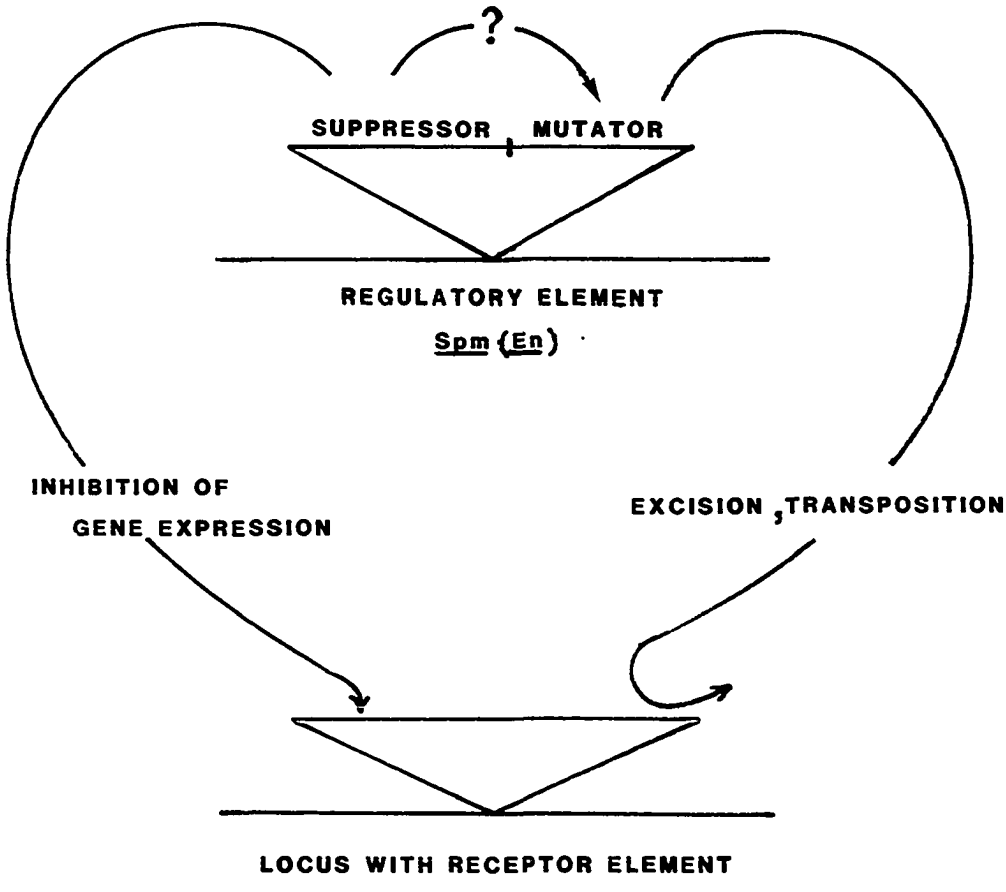

?
SUPPRESSOR , MUTATOR
REGULATORY ELEMENT
Spm (En)
INHIBITION OF
GENE EXPRESSION
EXCISION , TRANSPOSITION
LOCUS WITH RECEPTOR ELEMENT

Figure 2.3. Diagrams illustrating the origin of a newly broken end of a chromosome at the meiotic anaphase and its subsequent behavior (McClintock, 1951)

(A): (1) Homologous chromosome pairing; one chromosome carries a duplication in inverted order
(2) Crossing over results in dicentric chromatid
(3) Bridge configuration produced and break in the bridge occurs
(4) Fusion of sister chromatids at the position of break in (3)
(5) Separation of sister centromeres produces bridge configuration
(6) Fusion of sister chromatids at the position of break in (5)

A

The chromatid type of breakage-fusion-bridge cycle

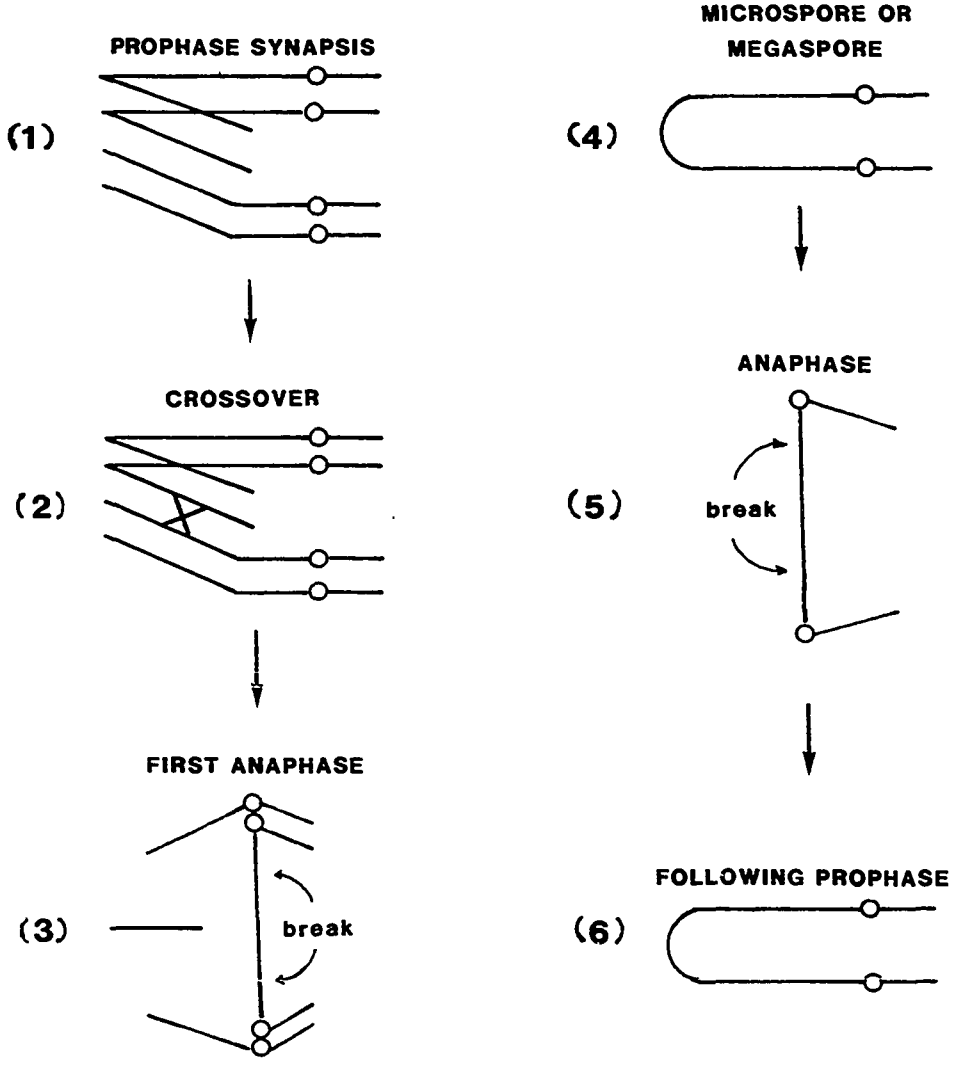
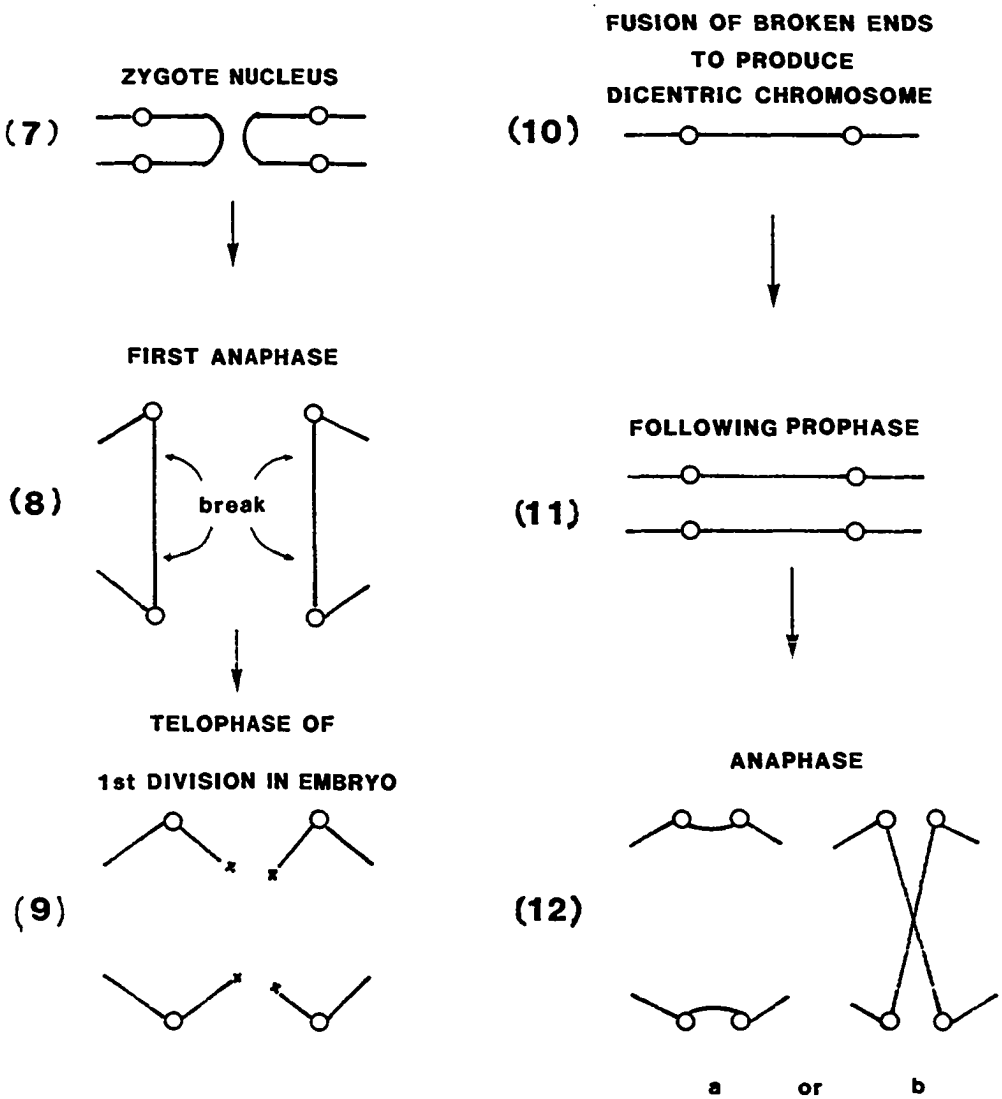


Figure 2.3. Continued

(B): (7) Fusion of the position of break
(8) Two chromosomes in (7) give rise to bridge-breakage; occurs in each bridge at some position
(9) Chromosome separation from (8); x indicates broken ends
(10) Fusion of broken ends of each chromosome establishing a dicentric chromosome
(11) Sister chromatid formation
(12) Configuration of separation from (11). Breaks occur in each bridge between centromeres. Subsequent behavior of the broken ends, from telophase to telophase is the same as that given in (9) to (12)

B

The Chromosome type of BFB cycle



nuclei, the fusions now occur between the broken ends of chromosomes rather than between the broken ends of sister chromatids. This cycle is illustrated in B of Figure 2.3.

McClintock (1978) reported that the BFB cycle represents only one mechanism that can trigger innate systems capable of inducing genome reorganization. The triggering results in various degrees of reorganization, ranging from complex chromosomal translocations to small segments of DNA, possibly composed of a relatively few base pairs.

McClintock (1978) described a series of expected chromosome aberrations such as duplications, reduplications in direct or reverse order, deficiencies, and translocations induced by the BFB cycle on chromosome 9 in maize. In addition to these expected aberrations, many unexpected chromosome modifications were noted. Some of the examples are listed as follows (McClintock, 1978):

(1) Fusion of centromeres of one chromosome 9 with that of another chromosome. An instance is shown in A of Figure 2.4.

(2) Fusion of the knob at the end of the short arm of chromosome 9 with a knob in another chromosome. An example appears in B of Figure 2.4.

(3) Fusion of the knob in the short arm of chromosome 9 with the centromere producing a ring chromosome. A diagram is shown in C of Figure 2.4.

(4) Knob-centromere inversion is diagrammed in D of Figure 2.4. It shows one chromosome 9 has lost all of its short arm. The homologue has an inversion that reverses the positions of the knob and the centromere.

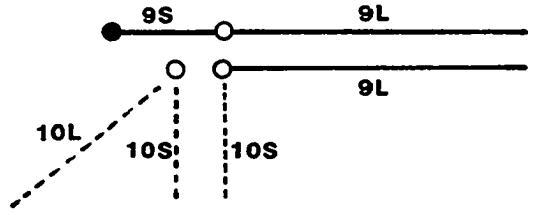
Figure 2.4. Diagrams showing different chromosome aberrations (McClintock, 1978)

○ centromere

● knob

● nucleolar organizer

A



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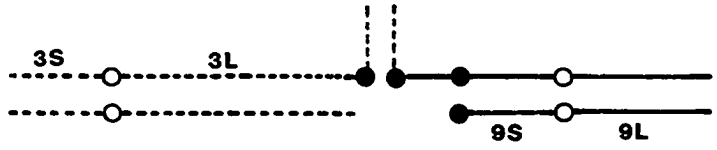

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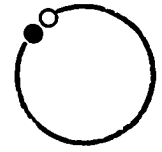
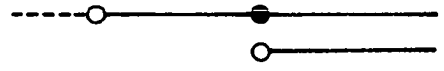
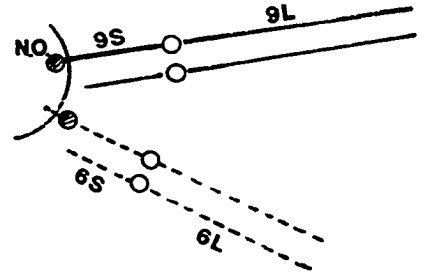


D



E



(5) Dicentric chromosome, produced by the end of the short arm of one chromosome 9 joined to a region just distal to the nucleolus organizer in chromosome 6. This is diagrammed in E of Figure 2.4.

McClintock (1978) also reported on the genetic and cytological analysis of the aberrations of chromosome 9 that were induced by an element. She termed this the x component and described it as follows:

(1) An x component is responsible for the chromosome rearrangements except the expected chromosome aberrations induced by the BFB cycle (Figure 2.3).

(2) This x component is located just proximal to the Bz locus and adjacent to or at the centromere of the fragment chromosome carrying the gene markers Yg C sh and bz.

(3) The x component induced modifications that restructured the fragment chromosome itself.

(4) The x component also is responsible for the observed variegation for the C Sh Bz markers on the chromosome 9.

(5) The x component showing some degree of specificity of action does not have any relationship with the controlling elements of the Ac, Spm, or Dt systems.

McClintock (1978) concluded that the components of transposable gene-control systems that previously were totally concealed may be readily triggered into action by the stress induced by the BFB cycle.

Another instance of the stress factor inducing the generation of transposable elements is the Dt system (Doerschug, 1973). He obtained two new Dt elements in the crosses of plants undergoing the chromatid

type of BFB cycle on chromosome 9 of maize and the a-m-1/a-m-1 or a/a plants.

Peterson (1983) stated that the induction of chromosome modifications represents one of the stress factors generating transposable element movement.

2.2.2. Specific mechanisms of chromosome rearrangement

2.2.2.1. Chromosome breakage at Ds The Ac-Ds system was discovered in maize lines undergoing a breakage-fusion-bridge (BFB) cycle within the short arm of chromosome 9 (McClintock, 1946). The two types of BFB cycle were illustrated in the Ac-Ds system by McClintock (1951).

It has been mentioned in Section 2.1 that chromosome breakage at the site of Ds insertion as well as transposition of the element requires the additional presence of the regulatory element Ac in order to activate chromosome breakage at Ds (McClintock, 1947).

In strains of the appropriate genetic constitution (see below), chromosome breakage at Ds is clearly manifested as a variegation pattern in the kernel. The kernel used to illustrate this phenomenon is heterozygous at C Bz and Wx loci having the constitution C-I Bz Wx Ds/C bz wx on chromosome 9 and also carries Ac. Chromosome breakage at Ds gives rise to an acentric fragment carrying the markers distal to Ds (C-I Bz and Wx) and a broken chromosome terminating at Ds (McClintock, 1947, 1948, 1951). The acentric fragment is lost at the subsequent mitotic division giving rise to sectors in the kernel that have only those markers proximal to Ds and on the unbroken homolog. Chromosome

breakage and the ensuing marker loss are shown graphically in A of Figure 2.5 (Fedoroff, 1983). Breaks occurred during the development of the kernel producing sectors that have the phenotype determined by the C bz wx homolog not carrying Ds. It was established that the three markers (C-I Bz Wx) are lost simultaneously as a consequence of chromosome breakage at Ds.

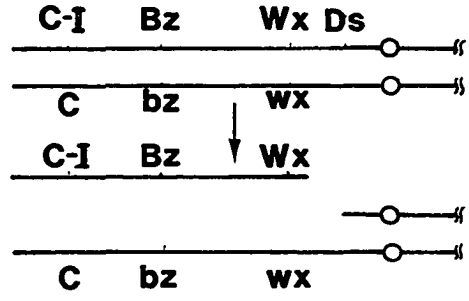
In cytological studies, it was established that the physical site of chromosome breakage correlated with its genetic position. Thus, the somatic loss of markers distal to Ds on chromosome 9 is correlated with the physical loss of the distal end of the chromosome (McClintock, 1947, 1951).

2.2.2.2. Acentric and dicentric chromosome formation at Ds

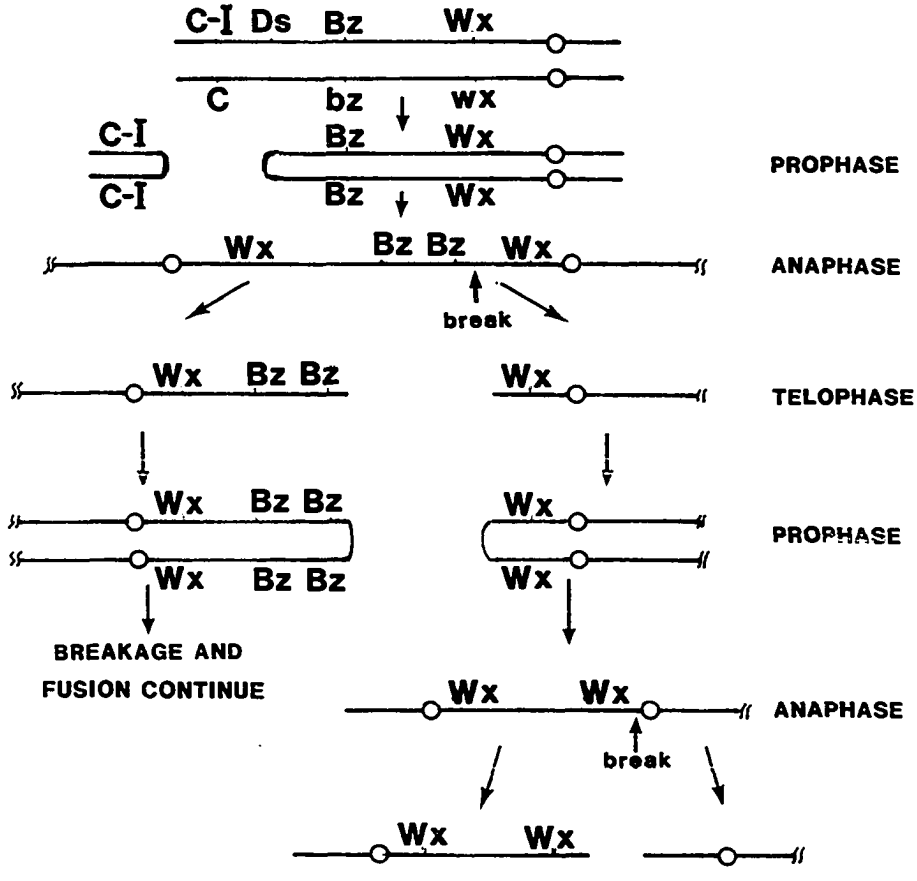
McClintock (1951) established that the chromatid-type of BFB cycle is induced by the effect of Ac on Ds on chromosome 9. The site of attachment of the chromatid fragment to each other coincides with the chromosomal position of Ds. In cytological and genetic studies it was found that the chromosome breakage at Ds in the presence of Ac is associated with the formation of an acentric fragment consisting of the two sister chromatids from the Ds position to the end of the short arm of chromosome 9 and a complementary dicentric chromosome including the two sister chromatids from Ds to the centromere plus the long arm of the two sister chromatids. An example illustrating this chromatid type of BFB cycle induced by Ds is shown in B of Figure 2.5. The genotype of the kernel is C-I Ds Bz Wx/C bz wx containing Ac. Acentric-dicentric chromatid between Bz and Wx results in the Bz duplication in one daughter cell and the deficiency of Bz in another cell. The descendants

Figure 2.5. A diagram showing chromosome breakage and acentric-dicentric formation at Ds
(A) Chromosome breakage
(B) Chromatid type of BFB cycle

(A)


C-I Bz Wx Ds
C bz wx
C-I Bz Wx
C bz wx

(B)


C-I Ds Bz Wx
C bz wx
C-I
C-I
Bz Wx
Bz Wx
PROPHASE
Wx Bz Bz Wx
ANAPHASE
break
Wx Bz Bz
Wx
TELOPHASE
Wx Bz Bz
Wx Bz Bz
Wx
Wx
PROPHASE
BREAKAGE AND
FUSION CONTINUE
Wx Wx
ANAPHASE
break
Wx Wx

of these two genotypically different daughter cells give rise to adjacent twin sectors in the kernel that are deeply pigmented (Bz Bz· Wx/C bz wx) and bronze (_ _ Wx/C bz wx) respectively. The BFB cycle continues to yield bz and wx subsectors in Bz Wx sector and wx subsectors in bz Wx tissue. The consequence of acentric-dicentric formation at Ds is shown graphically in B of Figure 2.5.

Other aberrations often arise. One of them is the formation of an internal deficiency in the short arm of chromosome 9. Such deficiencies include the regions adjacent to Ds. Translocation between chromosome 9 and another chromosome may arise with one of the points of breakage at the Ds position. Duplications or inversions of segments within chromosome 9 may also be produced, one of the breakage points being at Ds. This phenomenon will be discussed in the section on transposition of Ds (Section 2.3.1.1).

2.2.2.3. Spm(En) system on chromosome breakage The Spm(En) system differs from the Ac-Ds in several ways. One is the induction of chromosome rearrangements. Thus far, no systematic formation of acentric-dicentric chromosomes having the characteristics of Ds-induced BFB cycles has been observed in the Spm(En) system, though some Spm(En)-mediated chromosome breakage may occur (Fedoroff, 1983).

2.2.3. The molecular basis of minor chromosome aberrations induced by transposable elements in maize

According to McClintock (1978), the BFB cycle can trigger innate systems capable of inducing genome reorganization. The triggering results in various degrees of reorganization, ranging from complex

chromosomal translocation to small segments of DNA, possibly composed of a relatively few base pairs.

It has been previously discussed that the major gross chromosomal aberrations are especially obvious in the Ac-Ds system. Because of the rapid progress in molecular studies on transposable elements in maize, some of the results showing minor chromosome aberrations at the DNA level are presented here.

Sachs et al. (1983) sequenced the Ds at the Adh1 locus and found that the Ds insertion is AT-rich and is bounded by short (11 bp) inverted repeats. The insertion is flanked by direct 8 bp repeats which represent a duplication of base pairs 38-45 of the progenitor sequence. In the four revertants of Adh1-Fm335 mutant, Sachs et al. (1983) also found that the direct duplications which flanked the Ds insertion in the mutant were retained after the Ds insertion had been excised. However, there are changes in the sequence at the new junction of the duplicated segments. DNA strand inversion and base pair deletion were found in these four revertants. The diagrams showing the 8 bp repeats flanking the Ds insertion and the products of excision events in the four revertants are given in Figure 2.6.

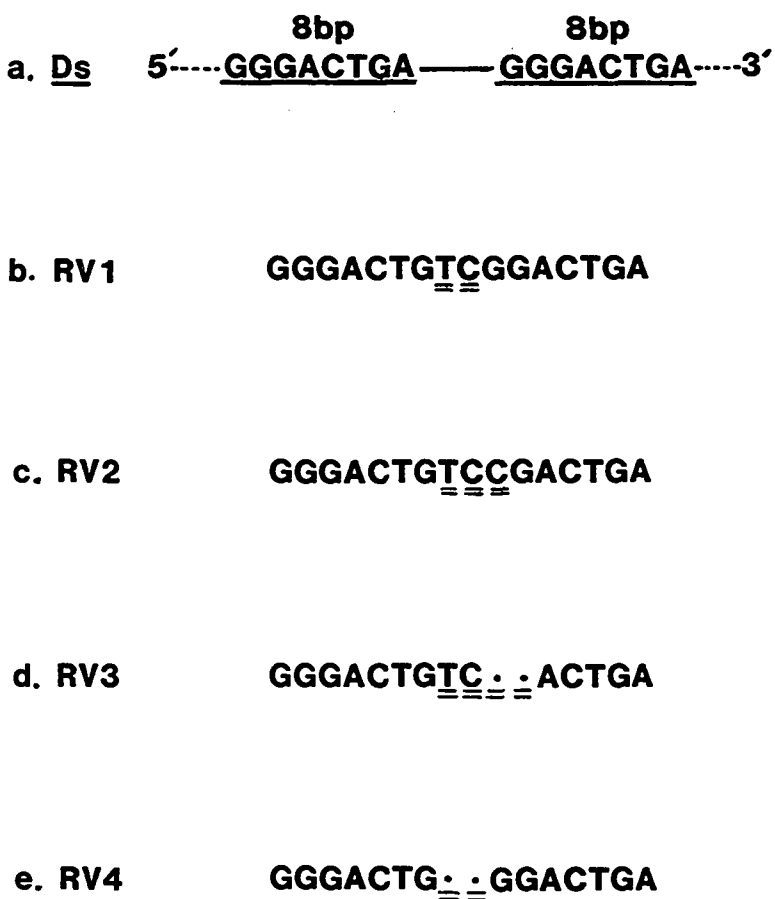
Döring et al. (1984b) isolated Ds from the sh-m5933 mutant and sequenced it. They also found that the Ds element in the sh allele terminates in inverted 11 bp sequence and is flanked by 8 bp repeats. They defined a DNA sequence having these characteristics as a transposable element. The 11 bp inverted sequence may be responsible for chromosome breaks and Ds transposition. The inverted

Figure 2.6. Nature of the direct repeats of Ds (8bp...8bp) and the revertants of Adh1-Fm335 (Sachs et al., 1983)

RV1 and RV2 show strand inversion at the junction

RV3 and RV4, in addition to strand inversion, there are 2 bp deletions

$\doteq$ denotes a deleted base and underlined bases are changes from those present in the original duplication


8bp
8bp
a. Ds
5'-----GGGACTGA——GGGACTGA-----3'
b. RV1
GGGACTGTCGGACTGA
c. RV2
GGGACTGTCCGACTGA
d. RV3
GGGACTGTC · · ACTGA
e. RV4
GGGACTG · · GGACTGA

11 bp sequence and 8 bp repeats of the Ds insertion at the Sh locus are shown in Figure 2.7.

Similar results have been obtained by Sutton et al. (1984) in the molecular analysis of Ds at the Adh1 locus. The results revealed that the 8 bp direct repeats flank the Ds insertion and 11 bp inverted repeat sequence at the Ds termini. When Ds transposes, the insertion with its inverted repeats is deleted, but the 8 bp direct repeats remain in modified forms. The sequences of different kinds of repeats of Ds insertion and the modifications on DNA sequence after Ds transposes are shown in Figure 2.8.

2.3. Transposition of Transposable Elements in Maize

In a previous section a general overview about the interaction of regulatory and receptor elements in two-element systems has been presented (Section 2.1). The receptor element is present at or near a gene locus where it suppresses gene activity when the regulatory element is located anywhere within the same genome. As the regulatory element acts on the receptor via some trans-action, excision and transposition take place. This is then followed by reinsertion of these elements into new sites on the same chromosome or on another chromosome, and this sequence of events results in variegation of gene expression.

In a one-element system, the loss of gene activity usually is accompanied by the absence of the transposable elements at the original locus and often by their reappearance at a new location. The transposition and reinsertion of these elements resulting in releasing and suppression of some gene activities should be observed.

Figure 2.7. The sequence of Ds at its termini (11 bp-inverted repeats (TAGGGATGAAA and TTTCATCCCTA) flanked by 8 bp-direct repeats (CGAAGTGG) in sh-m5933 (Döring et al., 1984b)

CGAAGTGGTAGGGATGAAA — TTTCATCCCTACGAAGTGG

Figure 2.8. Nucleotide sequences of the wild type progenitor (+31 - +50) and the terminal 11 bp inverted repeats flanked by 8 bp direct repeats of Ds in Adh1-Fm335. The 8 bp repeats represent a duplication of base pairs 38-45 of progenitor sequence (Sutton et al., 1984). (RV5A and RV10A showing strand inversion at the junction are exactly the same as RV1 and RV2, respectively, in Sachs et al. (1983).) The underlined bases are changed from those which are bracketed were present in the original duplications

a. wild type

31 38 45 50
CTGGTGAgggactgaGGGTC

b. Ds

gggactgaTAGGGATGAAA — TTTCATCCCTAgggactga
8bp 11bp 11bp 8bp

c. RV 5A

(AG)
GGGACTGTCGGACTGA

d. RV 10A

(AGG)
GGGACTGTCCGACTGA

The transposition of transposable elements in maize will be discussed in the following sections.

2.3.1. Ac-Ds system

2.3.1.1. Transposition of Ds

Ds was observed to change from its standard position, a few units proximal to the Wx locus, to a more distal position close to the Sh Bz and Wx positions, on the short arm of chromosome 9 (McClintock, 1951). Such transposition of Ds was recognized by the appearance of Ds-associated chromatid breaks at these new positions and the concomitant disappearance of breaks at the original position. Also with Ds at these new positions, some exceptional variegation patterns were derived.

The earliest example of transposition of Ds is the mutable allele c-m-1, and this allele expresses unstable recessive mutations caused by the presence of a Ds insert. This allele reverts to the C (color) phenotype in the presence of Ac, and therefore these kernels show colored spots on colorless background (McClintock, 1948, 1949). This new instability, c → C, was found to be associated with frequent chromosome breaks at the site which corresponded to the locus of C and with the disappearance of Ds-associated acentric-dicentric formation at the original site proximal to the Wx locus (McClintock, 1948, 1949). The disappearance of the breaking tendency from the region near the Wx locus and its simultaneous appearance at C, with the concurrent onset of genetic instability at or close to the C locus, is at least strongly suggestive of the transposition of Ds.

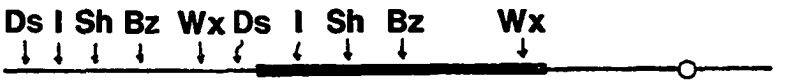
Transposition of Ds can also be accompanied by the production of chromosomal rearrangements which give rise to translocations, deletions, inversions, ring chromosomes, etc. (McClintock, 1948, 1949, 1950).

Two independent cases of chromosomal aberrations on the short arm of chromosome 9 were analyzed to illustrate the mode of transposition of Ds in the crosses of C sh bz wx/C sh bz wx (without Ac or Ds) x C-I Sh Bz Wx Ds/C sh bz wx (with one Ac, Ds at its standard position) (McClintock, 1950, 1951). From these crosses, two cases of duplication of a segment of the short arm of chromosome 9 appeared. The duplicated segment in the first case was in the inverted order; in the second case, in tandem order. A diagram showing the chromosome composition and genic order are given in Figure 2.9. There is revealed a new position of Ds activity that coincided with the position of one of the breaks that produced the duplication. Also, the position of the second break coincided with the previously known location of Ds in the morphologically normal Ds-carrying chromosome 9 of the male parent plant. It appears likely that the duplication accompanied the transposition of Ds that occurred during chromosome replication and resulted in the concomitant deletion of the corresponding chromosome region from sister chromatids (McClintock, 1950, 1951).

Ds is stable in the absence of Ac and, in some instances, Ds-altered expressions of the gene are not associated with release of Ds. The gene remains permanently under its control unless it is removed from the vicinity of the structural gene by crossing over (McClintock, 1965b). A rough map of the several sites on the short arm of chromosome 9 at

Figure 2.9. Diagrams (a) and (b) illustrate two different duplications of segments of the short arm of chromosome 9 and transposed Ds isolated and analyzed by McClintock (1950). The wide solid line represents the duplicated segment in each case. The original position of Ds was proximal to the Wx locus and coincides with one of the duplication end points in both cases. Transposition of Ds acccompanied the formation of the duplication in each case

(a)



(b)

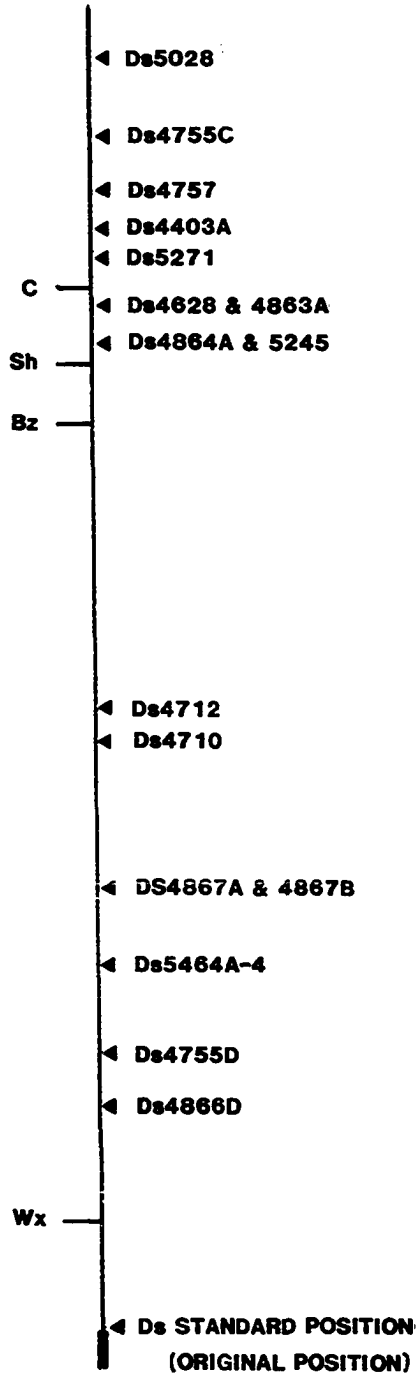
which Ds has been detected is given in Figure 2.10 (Fedoroff, 1983). In addition to sites shown in Figure 2.10, Ds has been identified as causing mutations at the C Sh Bz and Wx loci on chromosome 9 as well as at loci on other chromosomes (McClintock, 1948, 1951, 1952, 1953).

2.3.1.2. Transposition of Ac McClintock (1949, 1950) identified the transposition of Ac by using plants carrying Ds as males crossed onto F_2 plants containing Ac Ac. If in the F_2 plants containing Ac Ac no mutations occurred at the Ac locus or no transpositions have taken place, all the kernels should show the same pattern of Ds mutations. However, a small percentage of the kernels are markedly different. These exceptional kernels fall into three classes: (1) those that are completely void of Ds mutations, (2) those showing a few very late-occurring Ds mutations that suggest an increase in Ac dosage, and (3) those showing a time and frequency of Ds mutations that suggest a lower dosage of Ac.

Transposition of Ac will account for these results. It will produce (1) no Ac, (2) one Ac, or (3) two Ac in the nucleus depending on the transposition of Ac to nonhomologous chromosome or within the same chromosome.

Another example of an intially autonomously mutable allele capable of giving a two-element system is bz-m-2 with which Ac is closely associated (McClintock, 1956c, 1962). In the example, McClintock (1962) crossed plants carrying bz-m-2 to plants homozygous for the stable recessive bz yielded a range of mutant derivatives of bz-m-2 ranging in phenotype from dark to very pale. All the stable mutants tested, at

Figure 2.10. A map of the approximate position occupied by several Ds elements on the short arm of chromosome 9 (Fedoroff, 1983)


Ds5028
Ds4755C
Ds4757
Ds4403A
Ds5271
C
Ds4628 & 4863A
Ds4864A & 5245
Sh
Bz
Ds4712
Ds4710
DS4867A & 4867B
Ds5464A-4
Ds4755D
Ds4866D
Wx
Ds STANDARD POSITION
(ORIGINAL POSITION)

whatever level of expression, proved to have had Ac tranposed away from the Bz locus either to another site on the same chromosome or to another chromosome. In 33 new stable mutations, two of them showed Ac had been transposed to a closely linked site, 12 cases showed Ac had moved to a relatively distant site on the same chromosome, and the remaining 19 mutants showed Ac had moved to other chromosomes.

McClintock (1956c) found that in 16 out of 24 cases of stable recessive mutants from the bz-m2 allele, Ac was removed from the locus. Seven of these 16 cases were detected to show Ac was at a new location.

2.3.1.3. Mechanism of Ac transposition Critical evidence on Ac transposition occurring during chromosome replication is derived from studies with Mp. This is appropriate because Mp was shown to have properties indistinguishable from those of Ac when tested on plants carrying Ds (Barclay and Brink, 1954). Furthermore, of all the examples of transposition of a controlling element away from a gene locus, that involving P^{vv} has been the most informative.

The P^{vv} unstable allele was identified as a mutation resulting from the insertion of an element called Modulator of P (Mp) at the P locus (P^{vv} = P-Mp) (Brink and Nilan, 1952). P^{vv} shows a medium-variegated (red color on white) pericarp and cob. When Mp transposes to another location within the genome, the pericarp is fully red (Brink, 1958). When one Mp is located at the P locus and a second transposed Mp is present, the pericarp is lightly variegated (P-Mp + Mp) (Brink and Nilan, 1952).

Twin sectors of lightly variegated and fully red kernels are often found on the $\underline{P^{vv}}/\underline{p}$ heterozygote ears, suggesting that changes in Mp constitution occur (one Mp → no Mp or two Mp) at mitotic divisions, giving rise to two daughter cells that have lost and gained a copy of the element respectively (Greenblatt and Brink, 1962, 1963).

Removal of the element from its original site on one of the two chromatids is based on the results of the studies of the controlling element Mp. Two models were proposed to explain the movement of Mp (Brink and Williams, 1973; Greenblatt, 1974).

One model proposed by Brink and Williams (1973) is based on semiconservative DNA replication (Figure 2.11). In this model, replication triggers micro-nondisfunction of the Mp element. The single-stranded Mp element displaced from the chromosomes moved preferentially to a nonreplicated region on the same chromosome. The transposed Mp is copied in the sister strand by a DNA repair mechanism before replication in most cases.

Another model of Mp transposition is illustrated in Figure 2.12. This model (Greenblatt, 1974), based on conservative DNA replication, states that: (1) Mp replicates with the chromosome (Figure 2.12-2-(a)); (2) the newly replicated Mp, not the initial Mp, preferentially transposes to unreplicated sites on the old strand containing P-Mp (Figure 2.12-2-(b)); (3) Mp may replicate or may not replicate on the unreplicated sites with chromosome replication (Figure 2.12-2-(c)); and (4) sites are receptive to Mp insertion just prior to replication. This model was criticized by Fincham and Sastry (1974) because it depends on

Figure 2.11. A semiconservative chromosomal model illustrating transposition of Mp (Brink and Williams, 1973)
(A) Micronondisjunction of Mp during strand separation at DNA replication
(B) Initial incorporation in a single strand at the new site
(C) Tensional breaks occur in the other strand at the same level
(D) Repair

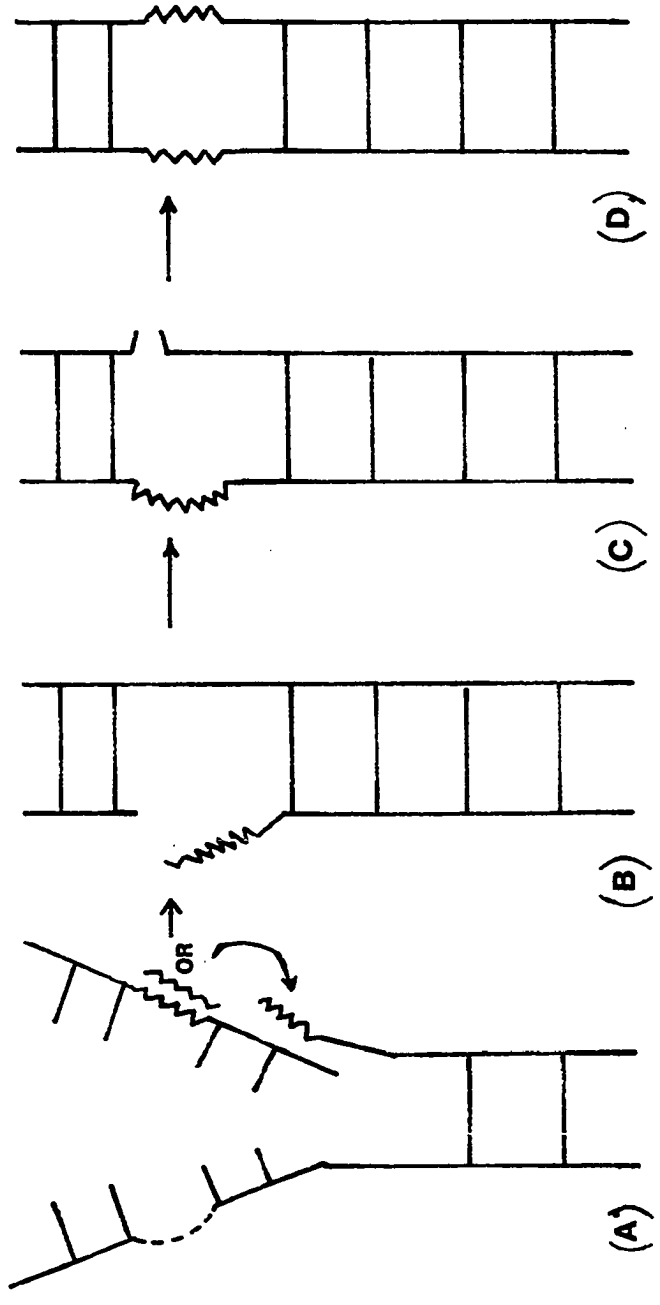

(D)
(C)
(B)
OR
(A)

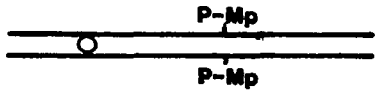
Figure 2.12. An outline of the transposition of Mp based on conservative replication (Greenblatt, 1974)

FR = Full red
LV = Light-variegated

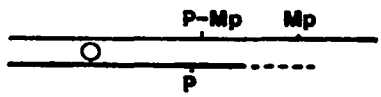
1 ELEMENT CONJOINED WITH CHROMOSOME

2 MITOTIC CHROMOSOME REPLICATION

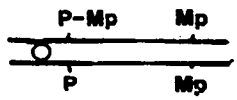
(a) replication of P locus and Mp



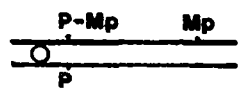
(b) transposition of new copy to old strand at an unreplicated site



(c) completion of replication



OR



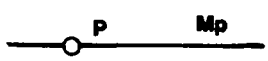
Mp replicate

Mp does not replicate

3 MITOTIC SEGREGATION

LV

LV



FR

FR

the assumption of conservative DNA replication, though this model can account for the results.

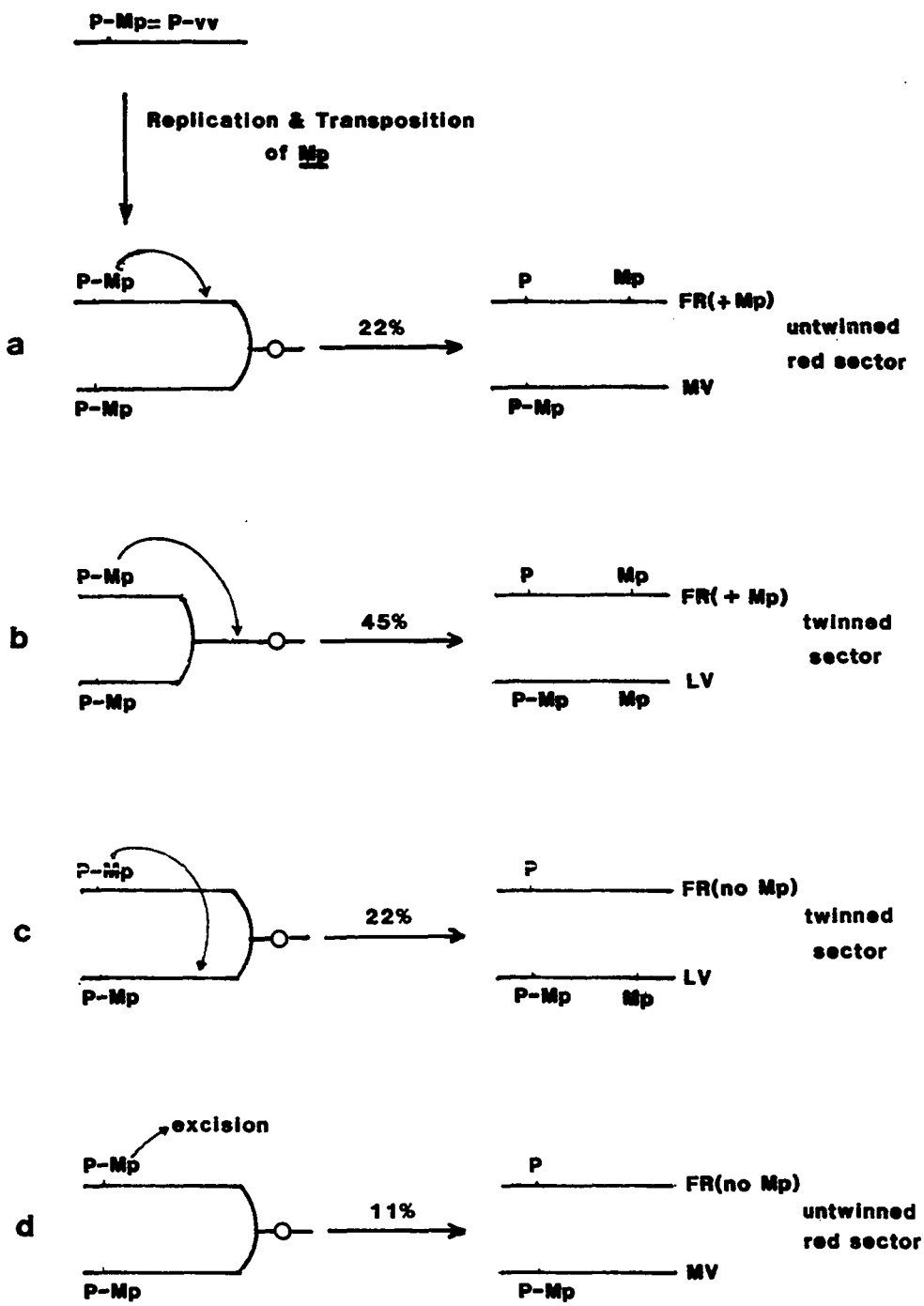
Both of these two models were made on two assumptions: (1) that DNA replication triggers transposition and (2) that the Mp element moves to an unreplicated area of the chromosome. Both models presume that twin sectors are an obligate by-product of transposition. However, this is not the case. The untwinned red and light-variegated sectors may be accounted for by the simple excision, intrachromatid transpositions, or replicative transpositions.

Fincham and Sastry (1974) proposed an alternative hypothesis. In their model, Mp is triggered to transpose by the process of replication of DNA and reinsertion of Mp independent of DNA replication based on the results from the twin sectors. They assumed that transposition of Mp occurs from a recently replicated P^{vv} (P-Mp) either to an unreplicated site most likely on the same chromosome or about equally frequently to an already replicated site; in a proportion of cases of transposition (about 10%), the Mp element is supposed to be lost or inactivated. Occasional transposition from an unreplicated P^{vv} is not ruled out. The diagram illustrating the model is given in Figure 2.13.

Recently Fedoroff (1983) described a very similar hypothesis of various types of intrachromosomal transposition of Mp with that of Fincham and Sastry (1974). She proposed that the twin sectors resulted from Mp transposition during or after replication of the Mp at the P locus and this results in the removal from the P locus on one sister chromatid and its insertion either in a replicated site on the other

Figure 2.13. Diagram illustrating the transposition of Mp with the replication of chromosome to produce twinned and untwinned sectors in the progeny of P^{VV}/p heterozygotes (modified from Fincham and Sastry, 1974)

FR = Fully red
LV = Lightly-variegated
MV = Medium-variegated


P-Mp= P-vv
Replication & Transposition
of Mp
a
P-Mp
P-Mp
22%
P
Mp
FR(+Mp)
untwinned
red sector
MV
P-Mp
b
P-Mp
P-Mp
45%
P
Mp
FR(+Mp)
twinned
sector
LV
P-Mp
Mp
c
P-Mp
P-Mp
22%
P
FR(no Mp)
twinned
sector
LV
P-Mp
Mp
d
excision
P-Mp
P-Mp
11%
P
FR(no Mp)
untwinned
red sector
MV
P-Mp

sister chromatid or in an unreplicated site on the same chromosome. The possibility that simple excision and other types of transpositions, including intrachromatid and replicative transpositions, also occur will explain the occurrence of untwinned red sectors (one Mp and no Mp) and lightly variegated sectors (two Mp). The diagram showing the different types of interchromosomal transpositions of Mp is given in Figure 2.14. Also, it is clear from the data that the interchromatid transpositions (Figure 2.14-a.b) are more prevalent than the intrachromatid transpositions (Figure 2.14-c.d) (cf Fedoroff, 1983).

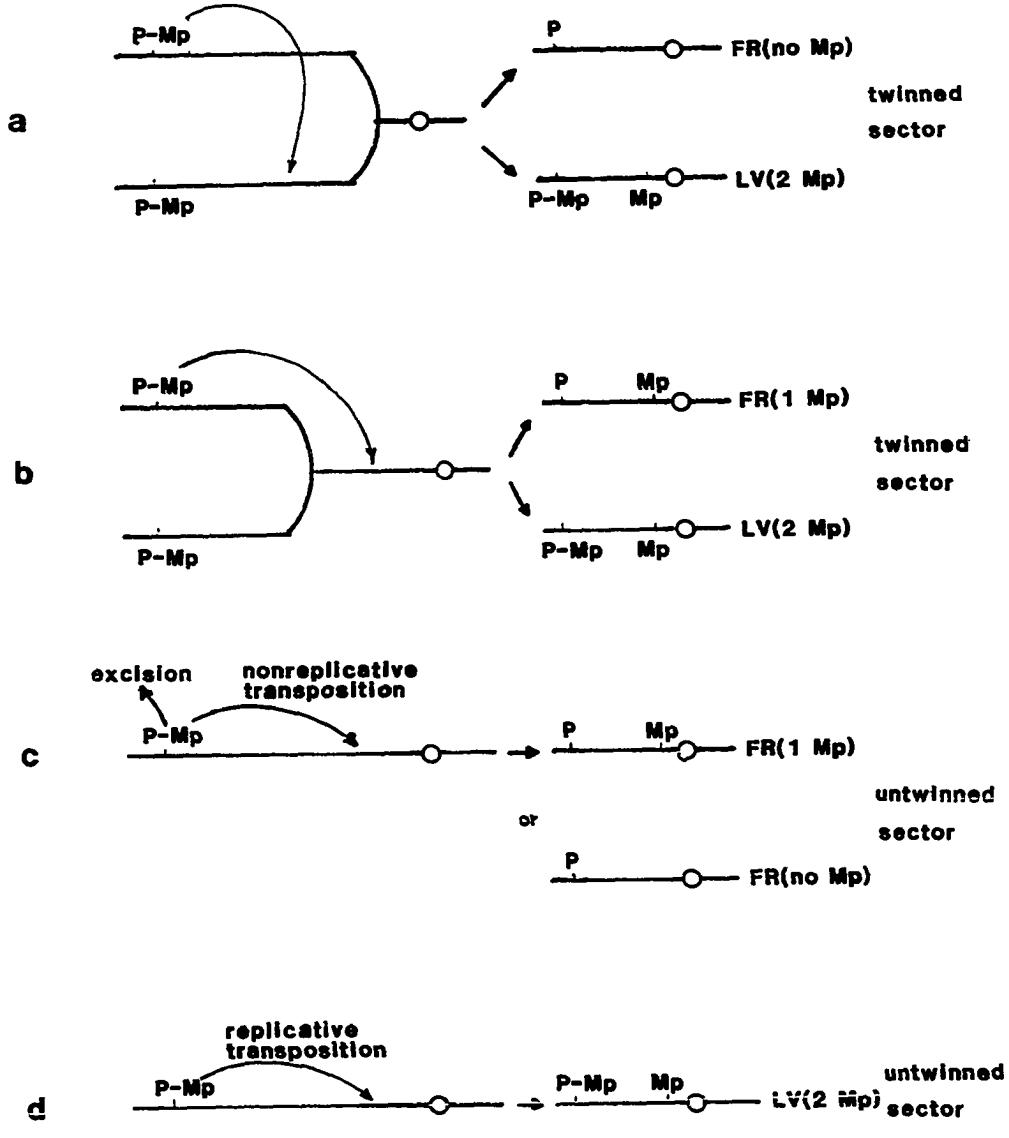
2.3.2. Spm(En) system

2.3.2.1. Transposition of Spm(En) In order to examine the constitution and location of Spm, McClintock (1956c) crossed a-m-1 Sh2/ a sh2 Pr/pr with one Spm linked with Pr as a female by a sh2/a sh2 Pr/pr or a sh2/a sh2 Pr/Pr. In the progeny of these crosses, she found that the constitution and location of Spm varied. Some plants had one Spm linked with Pr (chromosome) as their original parents. Some of the progeny had one Spm but not linked with Pr. Also, three Spm elements or no Spm elements were found in one plant. This showed the transposition of Spm from chromosome 5 to another place in the genome or its loss during the cell division.

Another example included the crosses of a-m-1/a-m-1 Wx/wx with one Spm linked to Wx and a-m-1/a-m-1 wx/wx (no Spm). McClintock (1956c) reported that the results obtained were similar to the results of the transposition of Spm from chromosome 5 to other places (McClintock 1956c).

Figure 2.14. A diagram showing various types of intrachromosomal transpositions. (a)(b) Transpositions occur during or after replication of the Mp at P locus result in the Mp removal from P locus (adapted from Fedoroff, 1983)

FR = Full red
LV = Light-variegated


a
P-Mp
P-Mp
P
FR(no Mp)
twinned
sector
P-Mp
Mp
LV(2 Mp)
b
P-Mp
P-Mp
P
Mp
FR(1 Mp)
twinned
sector
P-Mp
Mp
LV(2 Mp)
c
excision
nonreplicative
transposition
P-Mp
P
Mp
FR(1 Mp)
untwinned
sector
or
P
FR(no Mp)
d
replicative
transposition
P-Mp
P-Mp
Mp
LV(2 Mp)
untwinned
sector

In these tests, the Spm transposed premeiotically to a new location from chromosome 9 which allowed it to segregate at meiosis with the chromosome 9 carrying the wx allele.

Additionally, McClintock (1956c) found the constitution of Spm changed in the main stalk and tillers on the same plant. This also indicated the transposition or loss of Spm from its original site to a new site in the genome.

Furthermore, in the studies on the original a-m-2 at which the Spm was located, McClintock (1962) reported that there was a good correlation between transposition of the Spm element away from the locus and the production of stable mutants by using the data from the crosses of a-m-2 Sh2/a sh2 and a-m-1 sh2/a-m-1 sh2 (no Spm). Approximately 40% of the stable mutants analyzed had one or more Spm elements in the genome. Less than 10% of such mutants retained an Spm either at the locus or near by on the same chromosome, suggesting that transposition is generally accompanied by the removal of the element from its initial site to another site on a different chromosome.

In another study utilizing the mutable allele a-m(papu) in the En controlling element system, Peterson (1970) reported the transposition of the En element to a site on the same chromosome in 25% of the stable mutants tested. There is a preference for transposition to sites 6-20 units from a. Secondary transpositions of En occur approximately 12% of the time in one test to an independent position. Secondary transpositions take place to new linked sites. The data indicate that the transpositions can occur to both distal and proximal positions on

chromosome 3. Nowick and Peterson (1981) reported that En transposes from its initial site at the a locus to a primary site and then to secondary sites. All these transpositions occurred within the distal two-thirds of the 3L. maize chromosome arm from three autonomous mutable alleles of A. The distribution of En positions on the segment of chromosome 3 was not random and some regions of the chromosome were more likely to contain an inserted En.

2.3.2.2. Mechanism of transposition of Spm Following the analyses of the mutable allele a-m-2, a-m-5 with Spm and a2-m-1 with Spm-c (designated for an Spm that undergoes cyclic changes in activity (Fedoroff, 1983)) (McClintock, 1962, 1971), it has been established that the mechanism of Spm transposition has some parallels with the mechanisms of Mp transpositions.

Spm at the A1 locus (a-m-2 and a-m-5 allele) provides data that are consistent with a nonreplicative transposition mechanism analogous to that deduced for the Mp element (Fedoroff, 1983). Results of McClintock's (1962) study on a-m-2 and Peterson's (1970) on a-m-(papu) provided evidence for the nonreplicative transposition mechanism of Spm. The twinned sectors in the plants carrying both an a2-m-1 and a Spm-c element revealed the changes in Spm-c dosage. One-half of such a twinned sector shows a deeply pigmented phenotype in the absence of Spm, whereas the other half shows the variegation expressed when Spm-c dosage is increased (McClintock, 1971). These phenotypes resemble those of Mp on the P locus. It is reasonable that the twinned sectors arise

as a result of transposition of the element from only one of the two sister chromatids, followed by its segregation with the other sister chromatid at cell division (Fedoroff, 1983).

2.3.3. Limitations of controlling element transposition

McClintock (1956a) reported that 14 stable bz mutants were isolated from 16 bz-m2 mutants. Four of these 14 stable mutants showed that Ac was still linked with the genetic markers carried on the short arm of chromosome 9 after Ac transposed from the Bz locus. In one of these four plants, Ac was very closely linked with the Wx locus and, in the remaining three plants, Ac was close to the Bz locus. In another experiment, McClintock (1956c) found that 37.5% of the Ac transpositions from the Bz locus were found linked to sites on the same chromosome in 24 independent cases of mutation to a stable bz recessive. McClintock (1962) also found that among 33 examples of change of bz-m2 to an apparently stable null-expression allele, about 42% of Ac transpositions to a new location were linked to the markers on the short arm of chromosome 9. But on the basis of the inviability of kernels and plants induced by the Ds element at the new locations on the short arm of chromosome 9, McClintock (1956a) suggested that controlling elements may be inserted at a number of different positions which seem to be preferred. And these may represent only a selected number of possible sites at which the presence of the element does not induce inviability at some stage in development. This is because dominant lethality may be expressed when controlling elements are inserted at some positions in the

chromosome complement. Difficulties are encountered in attempting to determine whether a particular element can enter any site in the chromosome complement or is restricted to certain sites.

However, from the results on Mp transpositions, van Schaik and Brink (1959) demonstrated that there was a marked preference for sites close to P when Mp was transposed from the P locus. Out of a total of 87 different transpositions, 25 of these included an additional Mp that was not separated by crossing over from the P-Mp complex. In 13 cases, it was within five map units of P, and in another seven cases it was within 10 map units. This tendency of Mp to undergo short-range transposition was later confirmed by Orton (1966). Brink (1958) reported 65% of Mp transpositions were from P^{VV} to a linked region.

From the results obtained for the a-m(papu), Peterson (1970) showed that 25% of the En transpositions from a-m(papu) locus occurred to linked sites on the same chromosome. The results of Nowick and Peterson (1981) indicated the distribution of En positions on the chromosome 3 was not random, with some regions of the chromosome being more likely to contain an inserted En.

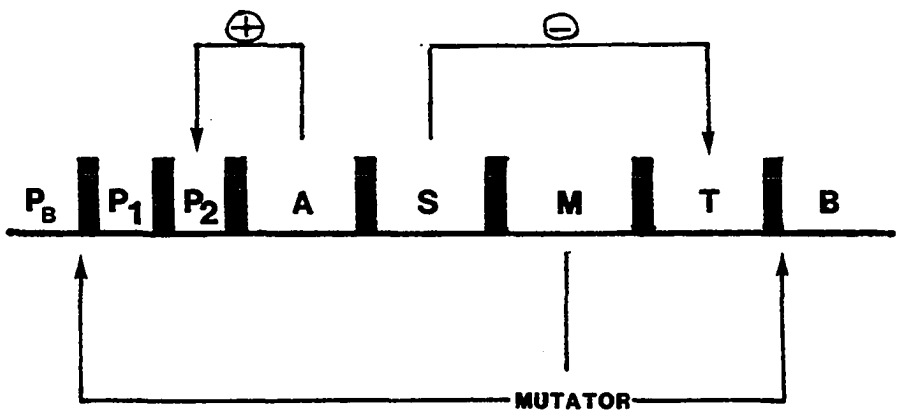
2.3.4. A model of the structure and function of a Spm element

A model of Spm(En) structure and function has been proposed by Nevers and Saedler (1977) based on the information obtained from bacterial transposons and insertion sequences (Figure 2.15).

According to this model, Spm contains two promotors (P1 and P2), three structural genes (A (activator), S (suppressor), and M (mutator)), and a termination sequence (T). S and M act independently in Spm.

Figure 2.15. Model for autonomous control of B gene by Spm(En) (modified from Nevers and Saedler, 1977)

B = a hypothetical gene
P_B = promotor of B gene
P1 = promotor of Spm that spontaneously starts transcription
P2 = promotor of Spm that intiates transcription only in the presence of A gene product
A = activator gene that induces P2 to start transcription
S = a gene suppresses transcription at the site of T
M = a gene excises at the black boxes indicated
T = a terminating sequence terminates transcription in the presence of S


⊕
⊖
P_B
P_1
P_2
A
S
M
T
B
MUTATOR

Spontaneous transcription starts at P1 and leads to the expression of the A gene. The A gene product is required for activation of P2 leading to the transcription of S and M genes. In an inactive Spm, the spontaneous transcription at P1 is not initiated if the P1 is in an inverted orientation, but the P2 of this inactive Spm which is still in direct orientation can be activated by the A gene product from an active Spm. In this way, an active Spm can cause trans-activation of an inactive Spm, as observed by McClintock (1968) at the a2-m-1 allele. The P1 and P2 inversion is proposed to control the cyclic activation and deactivation of the Spm.

In an autonomous system, the Spm is entirely integrated into the locus under control, e.g., the hypothetical B locus in Figure 2.15. The S gene product suppresses the B gene expression by terminating the transcription at T. The M gene product excises the whole Spm at the locus from the black box preceding P1 to the black box after T. The small black boxes are the sites of gene activity of the Spm model, thought to be similar to the IS elements. These are the sites of excision and integration at which the Spm components can undergo inversions, deletions, duplications, or transpositions resulting in various states of receptor and regulator on several mutable alleles (McClintock, 1951, 1967, 1968; Peterson, 1966, 1970, 1976).

Derivation of a two-element system from a one-element system (autonomous system) is explained by the excision of part of Spm leaving behind the T sequence bordered by two black box sequences. The residue (a receptor) can respond to the S and M gene products of an independently located Spm.

Because of change of S independently of M change in En element, Peterson (1981) modified Nevers' and Saedler's model. He put individual promotors for S and M components of En instead of one P1 for both. A changing S activity was accompanied by a constant M activity and vice versa on individual kernels containing a-m-1 alleles.

2.4. States of Controlling Elements

Diverse derivatives of a mutated gene are generated by a controlling element. These changes include a wide array of stable, phenotypically distinguishable, phenotypes that range from null to full expression. In addition, there are frequent changes to different heritable levels of mutability, which represent changes in receptor and/or regulatory elements.

The resulting patterns of mutability are distinguished on the basis of the timing and frequency of mutation events. Timing differences cause varied sizes of colored spots in aleurone tissue. Large sectors are caused by early mutations that contribute to a greater amount of tissue during ontogeny of the aleurone. Later-occurring mutations affect a small amount of tissue, resulting in smaller sectors in the kernel. Frequency is related to the number of mutation events which vary from a single spot to very frequent dense spots.

The combined effect of timing and frequency plus the level of gene expression in the mutant cell clones result in specific phenotypic patterns of mutability referred to as "states of the controlling element" (McClintock, 1951, 1955, 1968).

2.4.1. Ac-Ds system

2.4.1.1. States of Ac

2.4.1.1.1. Dosage effect of Ac

In early reports, McClintock (1948, 1949) found that, when more than one Ac element was present, the kernels showed sectors in which Ds breaks were more or less delayed and reduced in frequency, giving various grades of variegation. The same is true of acentric-dicentric formation which occurs later in the presence of more than one Ac element than it does in the presence of one element.

McClintock (1948) also found that the heritable changes affecting the timing of Ac-activated mutations give rise to a mutation pattern intermediate between those obtained with one and two doses of the parent Ac. She designated them as states of Ac.

2.4.1.1.2. Phase activity of Ac

McClintock (1964, 1965a) observed another type of change in which the instability in Ac underwent a reversible change between fully active and almost or quite inactive state of the Ac element. The study was facilitated by both the availability of wx-m-7 which has Ac at the Wx locus and the a-m-3 which has Ds at the A locus. Kernels carrying wx-m-7 showed striking variegation with respect to amylose starch. Some kernels, which from their parentage should have been of this type of wx-m-7, showed instead a low level of amylose. Within such kernels, occasional sectors were observed that had regained the typical wx-m-7 variegation. This reversible loss of mutability can be shown by the simultaneous action of Ac on the responsive a-m-3 allele. In the kernels in which wx-m-7 had become stable,

there was also an absence of a-m-3 mutations, while the endosperm sectors showing restored wx-m-7 variegation were precisely overlaid by sectors of a-m-3 spotting in the aleurone layer. The concerted behavior of the two mutable alleles showed that each was responding to changes in activity of the common controlling element Ac.

The reversible loss of mutability was due to a reversible inactivation of Ac (McClintock, 1965a). It was further established that the inactivation of Ac did not contribute to the Ac dosage effect and that the wx mutation could mutate somatically if an Ac element was introduced together with the inactive Ac at the locus (McClintock, 1964, 1965a). Changes of this type were designated as phase of activity. Reversible changes in phase have been extensively studied in Spm(En) system and will be discussed in later sections.

2.4.1.2. States of Ds In the studies of the mutable allele c-m-1, a Ds was inserted at the C locus. McClintock (1949) recognized that the Ds when first transposed to the C locus was one that produced many detectable chromosome breaks and few mutations for c → C. In another case, the kernels had sectors with high rates of mutation from c → C and a lower rate of detectable acentric-dicentric chromosomal formation. McClintock (1949) termed these two events as State I of Ds and State II of Ds, respectively. A series of derivatives that ranged from extreme State I to extreme State II depends on the various states of Ds.

2.4.2. Spm(En) system

Different states of controlling elements have been described by different phenotypic patterns of variegation. Because the mutations represent specific responses of the receptor element at the locus to an independently located element in a two-element system, a pattern of variegation is specified by the individual states of these elements. As with the Ac-Ds system, the states of receptor and regulatory elements in Spm(En) system will be discussed respectively.

2.4.2.1. States of receptor element A change in state of a receptor element occurs only in the presence of a regulatory element. In the absence of a regulatory element, the new state affects the level of gene action ranging from colorless to full colored.

In the presence of Spm(En), the original state of the receptor of the a-m-1 shows many early-occurring mutations (coarse spots) in the aleurone layer of the kernel. Eight distinctly different states of a-m-1 were tested in the presence of Spm(En). The range of their expression is from a few intensely pigmented spots in a colorless background of the kernel and a few streaks of deep pigmentation in the plant to a larger number of the same type of spots and with a number of fine streaks in the plant. In the absence of Spm(En), restricted gene action occurs and this results in plants that are darkly pigmented and the kernels are faintly colored. These expressions are constant in successive generations as long as Spm is absent. Control of all these mutabilities resides in the receptor element located at the A locus, although Spm is required for the manifestation of these controlled types of

expression. In other words, different states of receptor element of a-m-1 are responsible for the mutability (McClintock, 1955).

Peterson (1981) described different mutable alleles at the a locus. These include a-m(r), a-m-1, a-m-2, a-pale(mr), a-m(r)(flow), a-m(r)(crown), and these show the states of receptor element residing at A locus.

In the intensive study on five a-m mutable alleles of Spm(En) system, Reddy (1982) identified several changes in state of the receptor element at the A locus. The a-m-1(5719A-1) allele showed spotting pattern changes representing a change in state of receptor but without a change in the basic allele phenotype in the absence of Spm(En). The derivatives of a-m-1(6078) indicated changes in the state of the receptor with respect to its response to En and also in terms of the degree of suppression of gene activity at the A locus. In a-m-2(7977B), it was found that new states of the receptor element exhibited low response to Spm(En) and a variable level of gene activity (basic allele phenotype) in the absence of Spm(En). With the a-m-2(8004) allele, an exceptional kernel with t5 background of 1-2b-c spotted pattern is due to a change in state of the a-m-2(8004) allele; this change is most likely in that part of the receptor that responds to the suppressor component of Spm(En). In addition, another exceptional derivative of a-m-2(8004) allele which shows colored spots on a uniform pale background is also due to a change in state of the receptor of a-m-2(8004) allele.

Recently, support has been provided for some other attributes of the receptor element. The modified expression such as the number and

the size of spots, tissue specificity, and the level of gene activity on the background pigmentation are due to the change of the states of the receptor element in Cy-controlling element system (a new element Cy on Bz locus) (Schnable, personal communication).

2.4.2.2. States of regulatory element The Spm(En) element has two components, the suppressor (s component) suppresses the gene expression and the mutator (m component) induces mutations (McClintock, 1954). Alterations of one of these two components or both of them cause the changes in state of the regulatory element. For instance, in recent studies on the mutable alleles at A locus, Reddy (1982) reported that the exceptional derivatives of a-m-1(5719A-1), a-m-2(7977B), and a-m-2(8004) are due to the changes in states of the regulatory element in the Spm(En) system. These changes involve either the changes of m function or the changes of s function, which result in the changes of an original active Spm(En) element.

2.4.2.2.1. Spm^{S} and Spm^{W} Spm^{S} (standard Spm) is recognized by many deeply pigmented spots against a colorless background in a kernel with the a-m-1(5719A-1) allele. A new state of Spm shows a very late mutation and low frequency of mutation (one to several tiny spots of deep pigmentation) against a colorless background compared to Spm^{S}. McClintock (1957) called this state Spm^{W} (w for weak).

It is primarily the m function that differs between an Spm^{S} and Spm^{W} element. The mutator activity was weakened, and a few mutations were induced very late in endosperm development in Spm^{W}. However, McClintock (1957) observed that plants having an a-m-1(5719A-1) allele

and an Spm^{w} showed a deeper color than plants of the same genetic constitution but having an Spm^{s}. This observation implied that the S component is affected (weakened in potency) in some degree in Spm^{w} as well. Peterson (1966) noted a similar weak En which he designated En' by transposition of En from the a-m-flow allele.

2.4.2.2.2. Cyclic changes of phase activity The cyclic changes in phase of activity were observed in the class II state of a2-m-1 (McClintock, 1958). This state of a2-m-1 produces deep pigmentation in both kernel and plant in the absence of Spm. In the presence of an Spm, the pigment is produced in the plant, but it is less intense than that in the absence of Spm. However, in kernels, the pigmentation is completely suppressed by an active Spm element. This shows that this derivative of the a2-m-1 allele was unresponsive to the m function of Spm but still responds to the s function. The particular state of Spm present with this derivative of a2-m-1 is one that shows frequent inactivations during endosperm development, releasing the suppression of the a2-m-1 allele in clones of aleurone cells that appear as pigmented sectors within colorless sectors; often, colorless areas appeared. In some cases, the colorless areas in turn contained deeply pigmented spots. Such variegation is due to reversible cyclic changes of inactive → active → inactive of the Spm element. This state of Spm has a distinct dosage effect. The size and number of pigmented sectors of a2-m-1/a2 decrease with increasing number of this Spm element (McClintock, 1971). This dosage effect indicated that the Spm elements undergoing the cyclic changes in expression are independent of each other.

The cyclic changes of controlling element phasic activity have also been described for the En element (Peterson, 1965b, 1966). Peterson observed two different derivatives of the a-m(dense) allele in which En is closely associated with the A locus (Peterson, 1961). Each of these alleles showed specific tissue distribution of En action. One is a-m(crown), and in this allele the pigmented dots are concentrated in the crown of the kernel with only a few or no dots on the rest of the kernel. In the other allele, a-m(flow), and in contrast to the "crown" type, the spots are restricted to the basal portion of the kernel and no colored dots are seen in the crown. In studies with the heterozygote having the genotype of a-m(crown)/a-m-1 or a-m(flow)/a-m-1 with the En element, it became evident that the crown and the flow pattern are due to localized En activity according to the position of the cells or the time of their differentiation (Peterson, 1966). The endosperm of the crown of the kernel is thought to be differentiated at a different time than the rest of the kernel, and so the crown and flow states of En could be regarded as having been programmed to become active at these different times during development. As with the cyclic changes of Spm, both the m and s functions of En are coordinate in activity.

Another example of changes in phase of activity is noted with the components of the En regulatory element (Peterson, 1981). The phase change from S^u (unstable S) → s during the development of the kernel results in pale-colored spots or patches on the kernel containing the a-m-1 allele. The results were supported by cyclic changes of phase activity (S^u → s → S^u) independent of the m component, which remains unchanged in its activity.

2.4.3. Causes of changes in state

2.4.3.1. Composition hypothesis In her studies on Ac-Ds system, McClintock (1949) reported that there were two states of Ds at the C locus. A number of intermediate states ranging from State I to State II were also recognized (see section 4.1.2).

Although a derivative showing a high frequency of acentric-dicentric formation can mutate in a single step to one showing a low frequency, the converse is not true. The change from State II to State I requires several stepwise events, reflected in the intermediate states (McClintock, 1949, 1950). These observations suggested to McClintock that the individual states of the Ds are indications of the number of active Ds units that may be present in a small chromatin segment and that the change from one state to another involves a change in number and/or distribution of these units within the segment (McClintock, 1949).

In the Ac-Ds system, it has been known that the number of Ac in the nucleus controls the time and frequency of Ds mutations. The higher the doses of Ac, the later the occurrence of Ds mutations. Similar changes in the mutation response of Ds will be registered after a somatic mutation in a single Ac locus. These responses indicated that some quantitative change may take place at the Ac locus where it mutates, probably, to an increase or decrease in the number of subunits at this locus (McClintock, 1949).

2.4.3.2. Position hypothesis Peterson (1976) quantified the different patterns arising from the effects of En on a standard receptor allele a-m(r) and on the newly generated mutable alleles a2-m and c-m

(insertional mutations of En at the locus). These studies showed that there was no correlation between the pattern expressed by the regulatory element (En) before and after transposition. This result does not agree with the composition hypothesis of which En should exert a similar influence on the pattern of mutability irrespective of its position in the nucleus. The pattern of mutability, according to the composition hypothesis, from the insertion of a given element (e.g., En) at a new site should be predictable. Therefore, Peterson's finding (1976) on the a2-m and c-m mutable alleles supports the hypothesis that these diverse trans-effects of En on mutable alleles are not caused by a change in the genetic information of En itself but rather by its position. Peterson (1977) also described the position hypothesis on the studies of the a2-m allele that determines the states of mutability. Williams and Brink (1972) and Brink and Williams (1973) indicated that the diverse patterns of mutability expressed by 26 mR-nj alleles were caused by the different sites of Mp within the R locus. These studies also support the position hypothesis.

2.4.4. Presetting

"Presetting is a unique pattern of gene expression observed with certain Spm-responsive alleles that have been exposed to an active Spm element transiently during plant development" (Fedoroff, 1983).

McClintock (1963, 1964) found two derivatives of a-m-2, a-m-2(7977B) and a-m-2(7995), that exhibited the presetting phenomena. The original state of a-m-2 produces no color in the absence of Spm and gives a medium-high level of pigmentation with many mutant sectors of darker

or lighter pigment in the presence of Spm. However, the a-m-2(7977B) or a-m-2(7995) alleles continued to give an irregular mottled pattern of pigmentation among a few kernels, even after Spm has been removed by meiotic segregation. The results obtained by McClintock (1964) indicate that such patterns are detected only if the Spm is initially present in the same plant together with the a-m-2(7977B) or a-m-2(7995) alleles in early plant development. "Thus, the premeiotic exposure of either allele 'preset' it for later expression in the absence of the Spm element."

Further studies on "presetting" have revealed that the preset patterns are not heritable. The preset patterns are erased in subsequent generations with no permanent effects or changes on the locus (McClintock, 1964, 1965a). But "the preset pattern could be reestablished by a cross of the same type in which presetting was originally observed" (McClintock, 1963, 1964).

In addition, "the Spm^{W} and Spm^{S} elements are equally effective in presetting, but an inactive Spm is incapable of presetting either allele for later expression, suggesting that the s component of the element is responsible for presetting" (McClintock, 1965a).

2.5. Factors Affecting the Pattern of Mutability

Some internal and external factors that influence the patterns of mutability of unstable alleles rather than the transposable elements themselves have been studied and identified by several researchers.

The modifiers affecting the spotting patterns of kernels were found by McClintock (1957, 1958), Peterson (1976), and Reddy and

Peterson (1983) in Spm(En) controlling element system. The effect of temperature on the frequency of mutation of unstable alleles were studied by Rhoades (1941) on an a-Dt allele and Peterson (1958) on a pg-m allele. Plant vigor, plant development stages, and different plant tissues affecting the mutations in different controlling element systems were also studied respectively by van Schaik (1955), Peterson (1965b, 1966), and Fowler and Peterson (1978).

All of these factors will be discussed individually in the following sections.

2.5.1. Temperature

Temperature is the only environmental factor shown to have a strong effect on the mutable systems. Rhoades (1941) conducted an experiment with two different a-Dt strains. He studied the effect of different temperatures on the mutability of a, and the results showed a negative relationship between the temperature and the frequency of Dt-induced mutations in a. The higher the temperature, the lower the frequency.

Peterson (1958) studied the effect of temperature on the pg-m allele by exposing growing seedlings to two temperature regimes. He could measure the frequencies of mutation of pg-m to Pg, giving sectors of dark green on pale green, in seedlings raised at 28°C and at 16°C. He found 5 to 13 fold greater frequency at the higher temperature, and the effect of the temperature on mutation rate was independent of time. He also found the younger leaves showed higher mutation rate than those of the older leaves. This suggested that the more cell divisions exposed to

the differential temperature, the greater the opportunity for mutation to occur.

2.5.2. Plant vigor

van Schaik (1955) studied the effect of F_1 hybrids and the four component inbreds on the mutability of P^{vv} allele by measuring the mutation frequency of P^{vv} to P at three successive stages in plant development. She reported that the greater the vigor, the higher the mutability of the variegated pericarp allele (P^{vv}). Also, van Schaik (1955) compared the mutation rates in the normal-grown and stunted hybrids. She concluded that the stunted hybrids had a lower mutation rate than the well-grown material.

2.5.3. Main stalk versus tiller

Fowler and Peterson (1978) reported that one specific derivative of En, En-v with a2-m(papu), gave rise to some derivatives showing a very reduced expression (fewer mutant spots) in the ear derived from the main stalk of a corn plant. In contrast, the tiller ear showed an increased level of En-v expression (coarse pattern) compared with that of main stalk ear on the same plant. This increased level of mutability of the tiller ears can be maintained when transmitted through the main stalk ear in the subsequent generation. This is somewhat different from cyclic changes of phase activity. The tiller-inducing change of En-v indicates that the new phase is irreversible.

When the progeny of 10 main stalk ears were compared with their parental source, the heritability of the level of En-v was high from the

parents to their progeny main stalk ears. But the heritability of the level of En-v through the parental main stalk ears to the progeny tiller ear was low.

From these results, Fowler and Peterson (1978) proposed that heritable alterations of the En-v can be produced by endogenous environmental factors present during normal plant development.

2.5.4. Modifiers

From crosses a-m-1 Sh2/a sh2 containing one Spm with a sh2/a sh2, McClintock (1957) found one exceptional kernel exhibiting a much larger number of spots. From tests on this altered expression of a-m-1, it was found that this was due to an independently located element which was capable of markedly increasing the frequency of occurrence of mutation at a-m-1 when Spm was also present. This element modifying the frequency of mutation could undergo change of location in the chromosome complement in somatic cells. It behaves like a transposing element.

McClintock (1958) further described this dominant factor, called "modifier," which was able to restore full mutator activity when in combination with a weak Spm. The properties of modifier suggest that it represents a defective element that can be rescued by complementation between it and Spm^{w}. Modifier, in the presence of Spm, increases the rate of somatic mutation of the a-m-1 alleles that normally show a relatively low frequency of mutability, but can not increase the mutability of the a-m-1 alleles that normally show a high frequency of mutability.

Peterson (1976) reported that some exceptional kernels appeared at a low frequency with an alternative pattern with small spots. Among the

progeny of testcrosses of c-m(coarse) Sh/c sh and c sh/c sh, c-m(coarse) allele (En is located at C locus) shows a coarse mutability pattern and these exceptional kernels are identified as fine. A factor conditioning this change from coarse to fine was found, and it assorts independently of the c-m allele. The results show that this factor is not En. Because this factor restrains the coarse pattern, the factor is called Restrainer (Rst). Peterson (1976) suggested that Rst is similar to Modifier (McClintock, 1958) in its effect on mutability or to modifying factors in the dotted series (Rhoades, 1941).

Reddy and Peterson (1983) found a modified expression (coarse is modified to fine) in their study on the pattern differentiation of six c-m mutable alleles in En controlling element system. This modified expression is under the control of a factor, segregating independently. This factor is a modified En with an altered m function and thus delay the m function on the coarse allele resulting in the expression of fine pattern on the kernels. It functions similarly to a "modifier." This factor was designated En-malt. Reddy and Peterson (1983) proposed a replication-transposition hypothesis to illustrate the origin and function of En-malt.

Modifiers have also been identified in other controlling element systems (Rhoades, 1938; Ashman, 1959, 1960).

2.5.5. Plant development stages

This topic has been discussed in section 2.4.2.2.2. The phase changes of Spm(En) system are closely tied to plant development.

Peterson (1965b, 1966) observed two derivatives of a-m(dense) in which En is closely associated with the A locus (Peterson, 1961). One is called a-m(crown) and the other is a-m(flow). These crown and flow phenotypes are due to controlled changes in the activity of En according to the position of the cells or the time of their differentiation. The endosperm of the crown of the kernel is thought to be differentiated at a different time from the remainder, and so the crown and flow states of En could be regarded as having been programmed to become active at these different times during development.

2.6. Molecular Studies on Transposable Elements in Maize

2.6.1. General findings

Transposable elements were described in genetic detail by several researchers (e.g., McClintock, Peterson, etc.). The biochemical characterization of these elements would be desirable to give an insight on the nature of transposable elements in maize. An approach towards this goal would be the isolation of a gene, of which mutants are available that are caused by the insertion of one of these transposable elements within this gene or in its immediate vicinity. Comparison of genomic DNA from the wild type and the mutants could reveal the transposable element as DNA present in the mutant, but absent from the wild type at that particular gene.

The molecular analysis of the unique chromosomal plant genes requires cloning of these genes using in-vitro recombinant DNA technology. For this purpose, a gene specific hybridization probe is needed.

Often such a probe is a cDNA made from the mRNA of the specific gene, or it may be the same gene cloned from another species in heterologous hybridization. With the help of such a probe, one of the clones containing the structural gene could be identified and the insertional mutations induced by transposable elements might facilitate the identification of genes. In such mutants, the structure and the size of the wild type structural gene should be altered, and an alteration will be seen via heteroduplexing between the wild type and mutant genomic DNA fragment.

Three major genes with known enzymes for Adh1, Sh, and Wx are suitable for this molecular study.

2.6.1.1. Adh1 gene The Adh1 gene codes for the enzyme alcohol dehydrogenase, an abundant and easily detectable protein in the seed, in anaerobically induced roots, and in several other tissues, including pollen and the aleurone. Many mutants and variants of Adh1 have been studied biochemically and genetically (review Freeling and Birchler, 1981) and cDNA copy of Adh1 mRNA has been cloned and sequenced (Gerlach et al., 1982).

2.6.1.2. Sh gene This gene encodes endosperm sucrose synthase in large amounts (Chourey and Schwartz, 1971). The mutants of this gene that are caused by the presence of the transposable element Ds were isolated (McClintock, 1952, 1953). cDNA clones derived from the mRNA of the Sh gene have been isolated (Burr and Burr, 1981a, b; Geiser et al., 1980; Fedoroff et al., 1983a). The Sh gene, therefore, like the Adh1 gene, does provide a highly accessible system by which a cloned probe could be prepared and used to describe the transposable elements in maize.

2.6.1.3. Wx gene It has been known that the Wx gene in maize determines the amylose content of endosperm and pollen. It encodes a starch granule-bound UDP-glucose starch transferase in large amounts in the endosperm (Nelson and Rines, 1962; Tsai, 1974). Many mutations of this gene have been identified at which the transposable elements reside (McClintock, 1951, 1952, 1963, 1964). Recently, Shure et al. (1983) and Fedoroff et al. (1983a) cloned the Wx gene and several Ac-Ds induced mutation alleles. Schwarz-Sommer et al. (1984) cloned the Wx gene successfully and isolated the I element from the wx-m-8 mutable allele (I is at the Wx locus). These results provide a basis to study the transposable elements on the molecular level.

2.6.1.4. Miscellaneous studies Thus far, among the anthocyanin loci, only the UDP glucose:flavanol 3-O-glucosyl transferase in endosperm of maize controlling one of the steps in anthocyanin biosynthesis in maize has been purified (Dooner and Nelson, 1977), though many loci in the anthocyanin biosynthesis at which transposable elements are integrated are easy to study genetically. Therefore, studies on the transposable elements of the genes involving pigmentation production on DNA level is difficult in maize.

Recently, Wienand et al. (1982) used Petroselinum chalcone synthase cDNA to probe wild type and mutant DNA from maize. In this heterologous system, none of the maize genomic clones, based on comparable unstable genes and revertant genes, included the maize chalcone synthase gene. But this study did provide a general method to identify plant genes with an integrated element. Shepherd et al. (1982) successfully cloned a

genomic fragment carrying a 700 bp insertion element Cin1 from a Northern Flint Line by using the LC1 as a probe on an EcoR1 digest of a Northern Flint Line. The LC1 is one of the four maize clones rescued via a heterologous probe by Wienand et al. (1982). The Cin1 insertion element is present in multiple copies in both W22 (a midwestern inbred line) and Northern Flint Line (Shepherd et al., 1982).

2.6.2. Specific findings

2.6.2.1. Ac-Ds system

2.6.2.1.1. Ds at the Sh locus

It is known that the isolation of transposable elements in plants is very difficult by using conventional methods because gene products of transposable elements in plants have not been found. However, it is possible to isolate DNA clones of genes that have previously been mutated by the insertion of a particular transposable element. Isolation of DNA from wild type and mutant genes reveals the presence of DNA foreign to the gene which can then be identified as the DNA of the transposable element.

The first plant gene to be used in this way was that coding for endosperm sucrose synthase at the Sh locus. The mutants of Sh gene that are caused by the presence of the Ds element in its vicinity were isolated (McClintock, 1952, 1953). These mutants revert to the wild type in the presence of Ac.

Many molecular studies on the Ds at the Sh locus have been reported by several researchers.

Döring et al. (1981) isolated the DNAs from wild type Sh gene and from three mutants caused by the insertion of Ds at the Sh locus, sh-m5933,

sh-m6233, and sh bz-m-4. These DNAs were compared in a Southern blotting experiment by hybridization to the Sh-cDNA cloned in pBR322. The differences between the DNAs of the wild type and the mutants indicate the presence of an additional DNA at the Sh locus and/or DNA alterations that have occurred subsequent to the insertion of Ds. No major band appears in the DNA of the double mutant sh bz-m-4 indicating that all or part of the Sh gene is deleted. This Sh deletion is consistent with the genetic evidence obtained by Dooner (1981) and is similar with that obtained by Burr and Burr (1981a).

Döring et al. (1981) suggested that these mutations at the Sh locus did not arise by a simple insertion of Ds in or near the mutated genes. The hypothetical structures of sh-m5933, sh-m6233, and sh bz-m-4 were established by them. It is assumed that both the sh-m5933 and sh-m6233 alleles include a deletion extending from Ds to the vicinity of the mutated gene as the cause of the mutants.

Because of the limitation on the number of restriction enzymes used in the study of Döring et al. (1981), the insertion site, orientation and size of Ds remain to be tested. But the sh-m5933 and sh-m6233 alleles were supposed to be located near the 5' end of the Sh transcription unit by Döring et al. (1981).

Burr and Burr (1982) used the clone and subclones of wild type sucrose synthase mRNA to probe the Sh gene and constructed the restriction map of this gene. sh-m5933, sh-m6233, sh-m6258, and sh-m6795 Ds-induced mutants of the Sh gene were also analyzed and partially mapped within the wild type locus. These four sh mutants have a common genetic

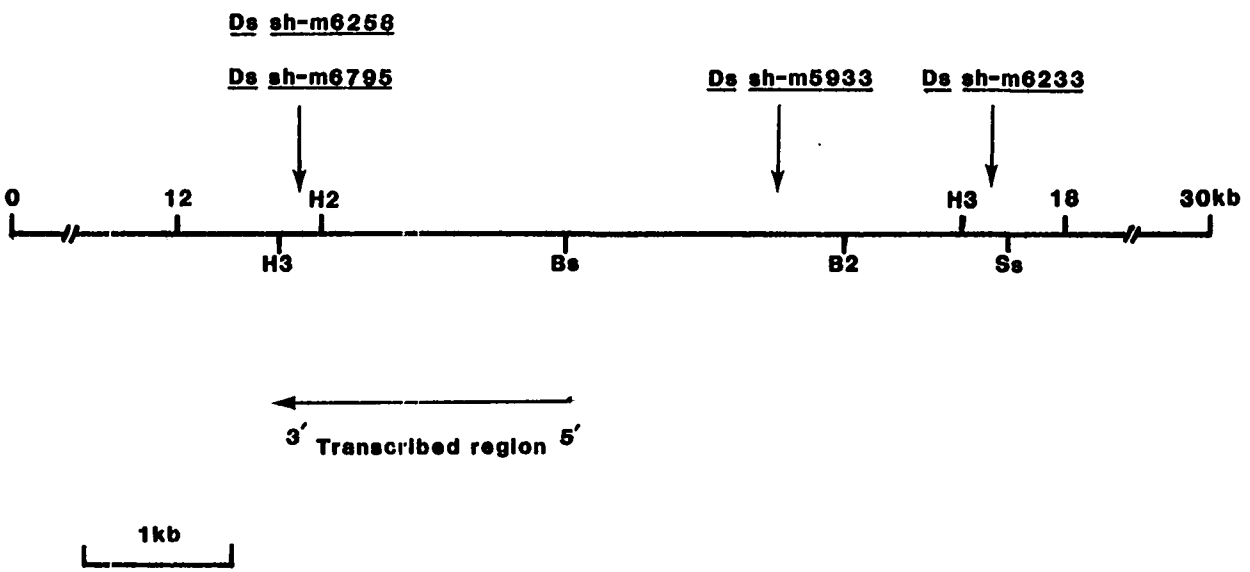
origin and are separated by only a few generations (McClintock, 1953, 1956b; Burr and Burr, 1982). sh-m6258 and sh-m6795 were found to contain an insertion of 21.7 kb of foreign DNA into the Sh transcribed region. The Ds element in sh-m6795 was found to be in the same site and oriented in the same direction as the Ds insertion in sh-m6258. Both of them were located within the transcribed region between 0.7 kb Hind III fragment and the 4.8 kb Hinc II fragment at the 12.8 kb on the scale (Figure 2.16).

The other two sh mutants with Ds insertions, sh-m5933 and sh-m6233, were placed at the 5' end of the transcribed region by Burr and Burr (1982). The Ds in sh-m5933 was positioned between Bst EII site at 14.6 kb and the Bgl II site at 16.5 kb. The sh-m5933 lies just outside the transcribed region (Figure 2.16). This Ds insertion is about 22 kb in length. However, Burr and Burr (1982) could not estimate the size of Ds in sh-m6233 because of less interpretable data for sh-m6233 in their study. But the Ds insertion was localized to a region to the right of Ds in sh-m5933 (towards the 5' end) on the map between the Hind III site at 17.3 kb and the Sst I site at 17.6 kb.

Burr and Burr (1982) also showed that there were profound differences in the restriction maps of these four sh mutants analyzed. They compared the restriction maps of two closely related Ds elements, sh-m6258 and sh-m6795 (sh-m6795 was derived from sh-m6258), and showed that five of the restriction sites are shifted 0.2 to 0.5 kb when made in side-by-side determination of these two Ds insertions. This may be the result of internal rearrangements such as small inversions. These

Figure 2.16. Positions of four sh mutable alleles on the restriction map of Sh (modified from Burr and Burr, 1982)

B2 = Bgl II
BS = Bst EII
H2 = Hinc II
H3 = Hind III
Ss = Sst I


Ds sh-m6258
Ds sh-m6795
Ds sh-m5933
Ds sh-m6233
0
12
H2
H3
18
30kb
H3
Bs
B2
Ss
3′ Transcribed region 5′
1kb

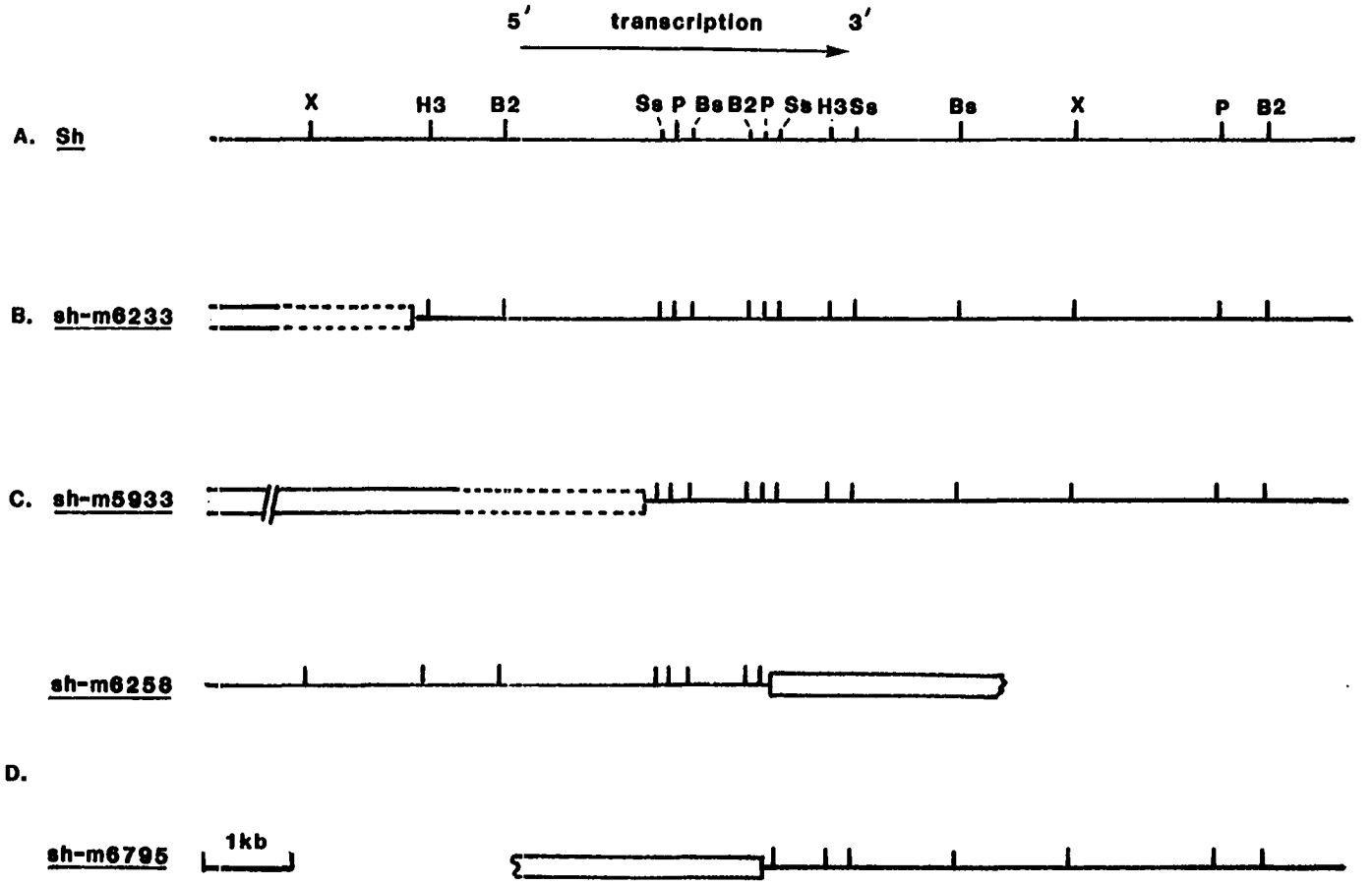
modifications are likely to be associated with events such as transposition or change of states (Burr and Burr, 1982). A revertant of sh-m6258 containing a 21-22 kb insert at the same location in the transcribed region was found. This Ds insert differed from its predecessor by extensive rearrangement in two-thirds of its length. Burr and Burr (1982) presumed that part or all of this rearrangement allowed the expression of Sh gene. No altered flanking restriction sites were found in these four sh mutations, though the Ds can clearly cause adjacent deletions (Burr and Burr, 1981a; Dooner, 1981; Döring et al., 1981). Therefore, Burr and Burr (1982) suggested that the Ds-induced mutations in these four sh mutable alleles are the result of simple insertions of large sequences into the Sh locus.

Fedoroff et al. (1983a) extended the findings of Döring et al. (1981) and Burr and Burr (1982). They constructed a limited restriction map of the Sh locus by using the cDNA insert from pSh8-1 as a probe. Four sh mutable alleles, sh-m5933, sh-m6233, sh-m6258, and sh-m6795, were also analyzed and mapped by blotting hybridization and restriction enzymes. The restriction maps are given in Figure 2.17. It indicates that:

(1) sh-m6233: The Ds element is located at the left end of the restriction map near the 5' end of the transcription unit. The point at which the restriction map of the sh-m6233 diverges from that of the Sh is located between Xba I and a Hind III site. But the precise location of the Ds endpoint in this region is uncertain. It may lie a kb or more 5' to the endpoint of

Figure 2.17. Limited restriction maps of Sh and four sh mutable alleles. The open boxes represent the insertion regions in sh-m alleles. The discontinuous portion of the boxes in B and C represents the uncertainty in the location of the junction between Sh locus sequences and foreign sequences (Fedoroff et al., 1983a)

B2 = Bgl II
H3 = Hind III
P = Pst I
Ss = Sst I
X = Xba I


5′
transcription
3′
A. Sh
X
H3
B2
Ss P Bs B2 P Ss H3 Ss
Bs
X
P
B2
B. sh-m6233
C. sh-m5933
sh-m6258
D.
sh-m6795
1kb

the Ds in sh-m5933. The breakpoints of the Ds elements in sh-m5933 and sh-m6233 were not known to lie within or outside the 5' end of the transcription unit.

(2) sh-m5933: Ds element is also near the 5' end of the Sh transcription unit, but the Ds endpoint lies somewhat farther toward the 3' end of the Sh locus than does the Ds in sh-m6233.

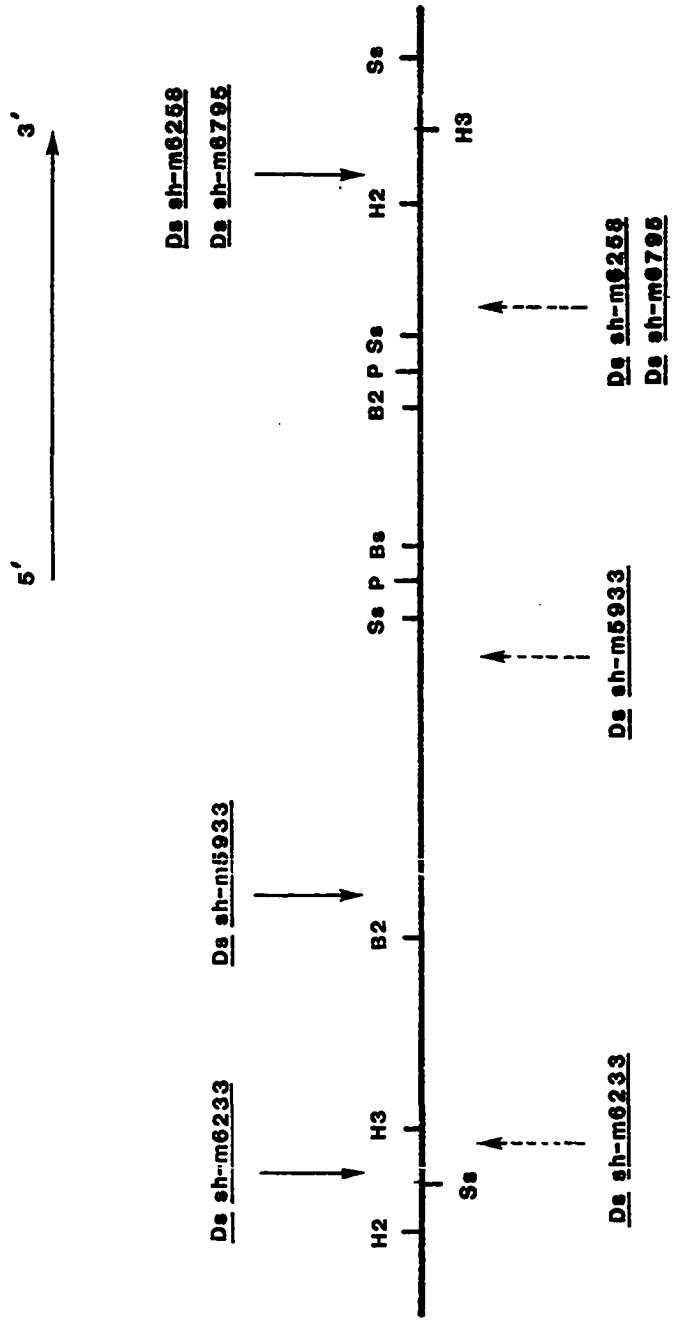
(3) sh-m6258 and sh-m6795: Both of the endpoints of the Ds elements of these two mutations lie within the Sh locus near the 3' end of the transcription unit. They were mapped at the same site. But these two Ds elements differ either in sequence or orientation in both sh-m6258 and sh-m6795 over parts of its length. It is suggested that these two sh mutable alleles arise by rearrangements such as inversion. Similar conclusions were obtained by Burr and Burr (1982).

The insertion sites of the four sh mutable alleles are similar, both in the observations of Burr and Burr (1982) and Fedoroff et al. (1983a). The relative positions of these four Ds elements are summarized in Figure 2.18.

Geiser et al. (1982) cloned the Ds DNA from sh-m5933. They constructed the restriction map of the sh-m5933 and found that the fragments cloned from Sh and sh-m5933 share a segment 6 kb long, while a contiguous segment of 15 kb of sh-m5933 does not hybridize to the DNA segment cloned from the Sh gene. But they could not conclude that the DNA found in the mutant but not in the wild type clone has been brought there by Ds insertion or by another Ds-dependent DNA rearrangement. Two pairs

Figure 2.18. Comparison of the relative positions of the four sh-m alleles on the restriction map of Sh between Burr and Burr (1982) (↓) and Fedoroff et al. (1983a) (↑)

B2 = Bgl II
Bs = Bst EII
H2 = Hinc II
H3 = Hind III
P = Pst I
Ss = Sst I


5'
3'
Ds sh-m6258
Ds sh-m6795
Ds sh-m6233
Ds sh-m5933
Ds sh-m5933
Ds sh-m6258
Ds sh-m6795
Ds sh-m6233
H2
H3
Se
B2
Se P Bs
B2 P Se
H2
H3
Se

of inverted repeats with several hundred nucleotide pairs in the DNA of sh-m5933 were found, one of which is located at the junction to wild type DNA sequence (Figure 2.19).

Though it has been discussed that the sh-m5933 allele is interrupted either by a long insertion or by some other type of rearrangement such as inversion, Courage-Tebbe et al. (1983) concluded that the structure of the sh-m5933 is somewhat more complex and presented the evidence as follows:

(1) The Sh locus is interrupted by a 30 kb insertion in the sh-m5933 allele. This insertion contains the genetically defined Ds element and falls within the Sh transcription unit.

(2) A partial duplication of unknown length that includes the 5' junction fragment between the Sh locus sequences and the insertion. Both copies of the partially duplicated locus are on the same chromosome, but the distance between them and the relative orientations are not known (Figure 2.20).

(3) All nine revertants analyzed have a structurally normal Sh locus lacking the insertion, but retain the partial duplicate 5' junction fragment presented in the sh-m5933 allele on the same chromosome.

(4) One of these revertant alleles, Sh-r5, comprises a normal or nearly normal mRNA coding sequence and also retains a copy of the duplicate 5' junction fragment, but about 2 kb DNA sequence is deleted at the junction between the Sh locus sequence and the duplicated portion of the insertion.

Figure 2.19. Restriction maps of Sh and Sh-m5933. The direction of transcription (5'→3') is from left to right. ← → and ←···→ are the two inverted repeats (Geiser et al., 1982)

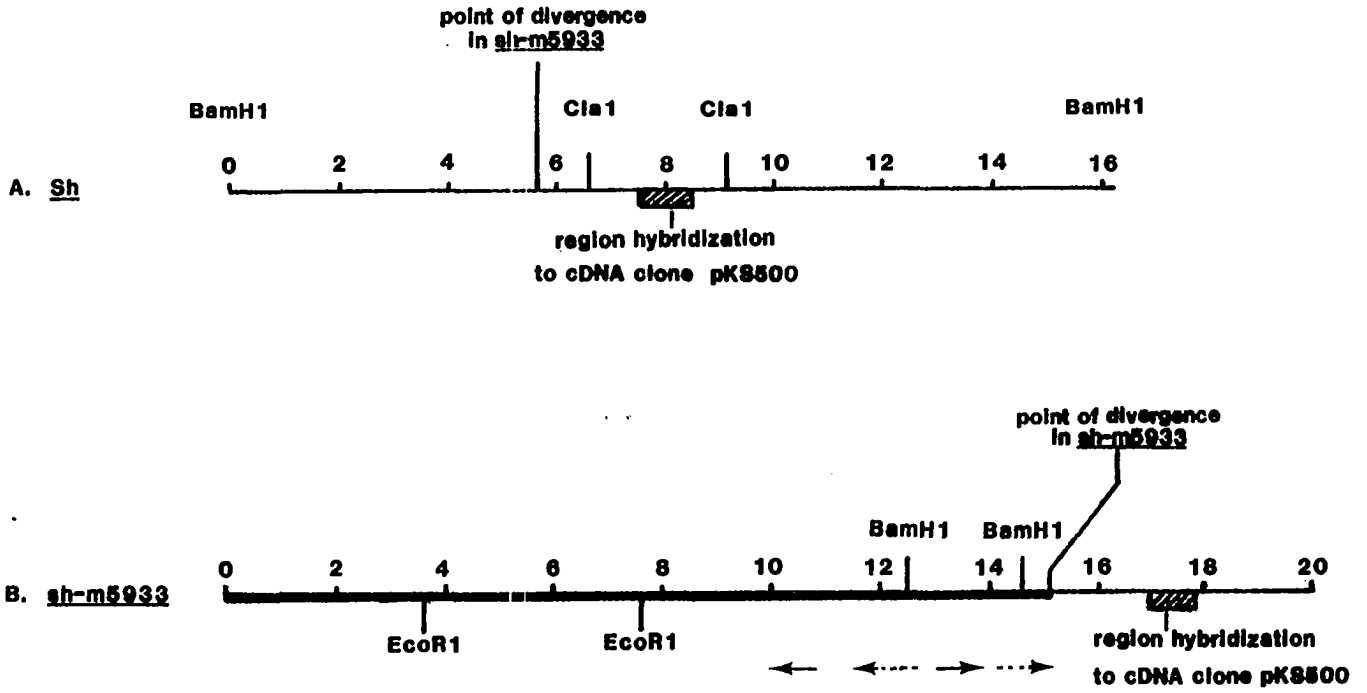
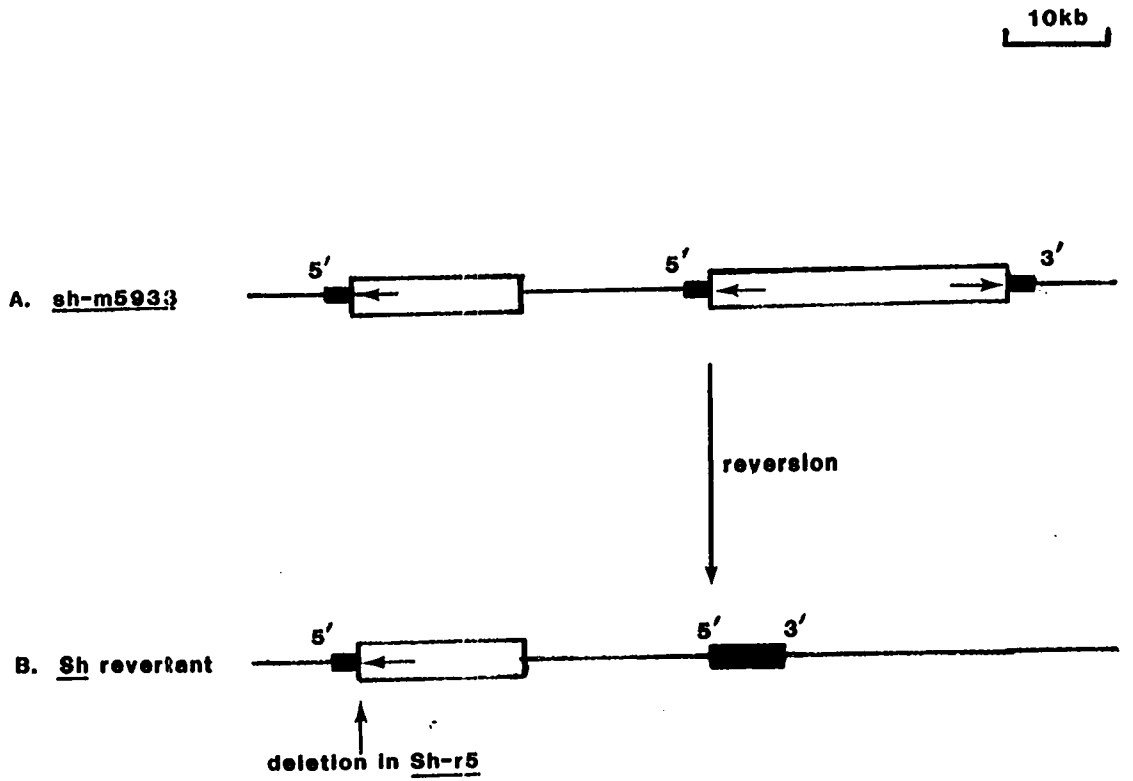

point of divergence
in sh-m5933
BamH1
Cla1
Cla1
BamH1
0
2
4
6
8
10
12
14
16
A. Sh
region hybridization
to cDNA clone pKS500
point of divergence
in sh-m5933
BamH1
BamH1
0
2
4
6
8
10
12
14
16
18
20
B. sh-m5933
EcoR1
EcoR1
region hybridization
to cDNA clone pKS500

Figure 2.20. A diagram representing the proposed structure of the Sh locus of the sh-m5933 and revertants derived from it (Courage-Tebb et al., 1983). Solid box represents the sucrose synthase coding sequence at Sh. Open boxes represent the foreign DNA introduced by the rearrangement in the sh-m5933. The details are discussed in the text. The deletion in Sh-r5 is that a sequence present adjacent to the 3' breakpoint is missing at the 5' breakpoint


10kb
A. sh-m5933
5′
5′
3′
reversion
B. Sh revertant
5′
5′
3′
deletion in Sh-r5

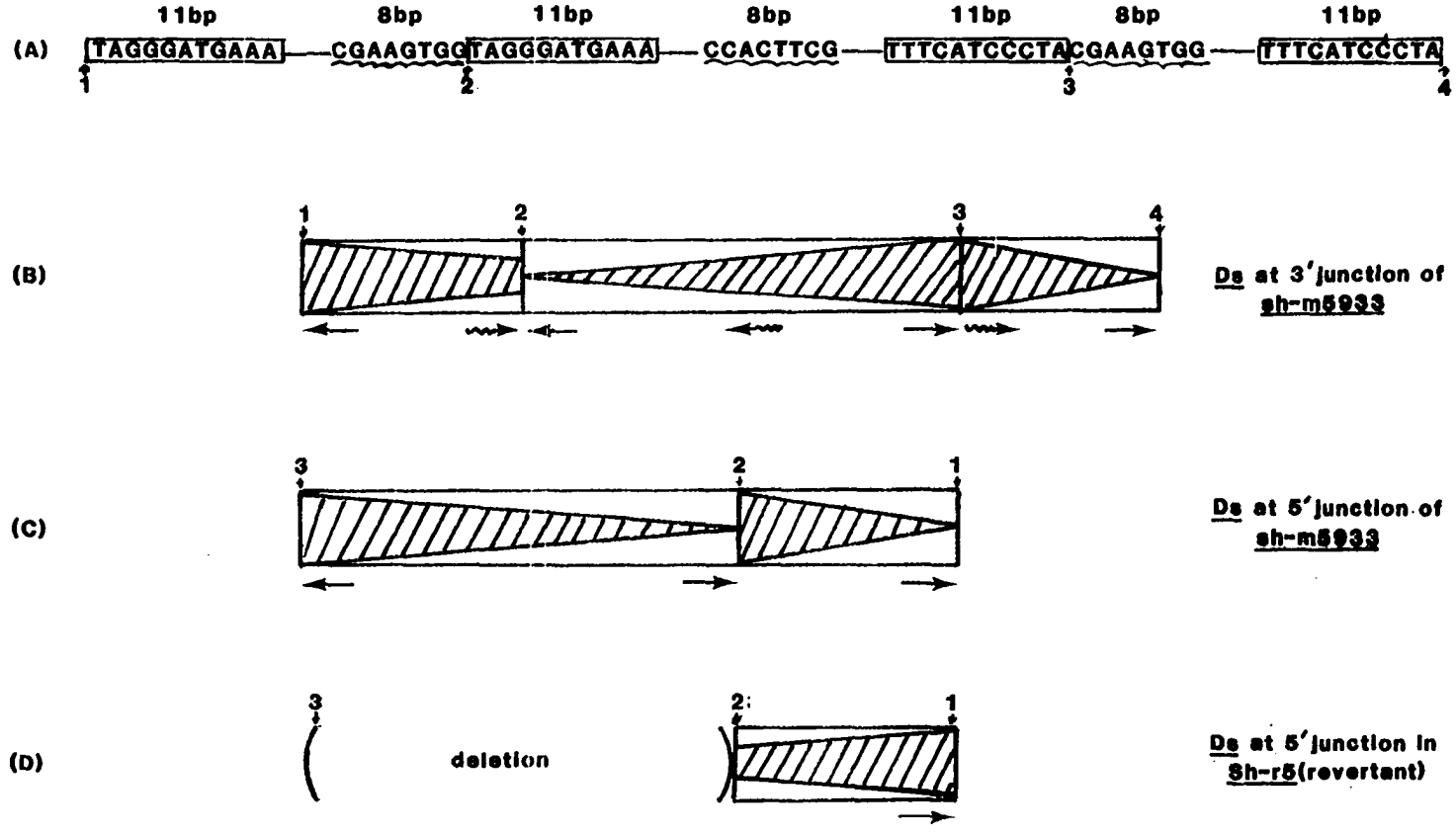
(5) The revertants continue to show Ds-mediated chromosome breakage at the Sh locus.

(6) The restriction map of the sh-m5933 is significantly extensive to rule out the possibility that this mutable allele contains a simple insertion of 22 kb, as reported by Burr and Burr (1982). Additionally, the entire 30 kb insertion of sh-m5933 does not correspond to the Ds element.

More details on the structure of the Ds element in the sh-m5933 allele were investigated by Döring et al. (1984b). They extended the findings of Geiser et al. (1982) and Courage-Tebb et al. (1983). The results of Döring et al. (1984b) show that the first 4.2 kb of the 30 kb insertion in the Sh gene of sh-m5933 comprises two identical 2040 base pair segments, one inserted in the reverse direction into the other. This 2040 bp sequence of DNA is a Ds element. Both the inserted and the recipient copy of this 2 kb sequence terminate in 11 bp-inverted repeats. This 11 bp sequence is found four times, the two right-hand and the two left-hand ones being oriented in the same direction with respect to each other. The internal 11 bp inverted repeats are flanked by 8 bp direct repeats (Figure 2.21A). These findings are compatible with the results from Sachs et al. (1983) and Sutton et al. (1984) which also showed that 11 bp-inverted repeats are present at the termini of Ds element in Adh1-Fm335 and are flanked by 8 bp-direct repeats. The 11 bp at the termini of the 2 kb transposable element in sh-m5933 is identical to the 11 bp-inverted repeats described by Sachs et al. (1983) and Sutton et al. (1984) in Adh1-Fm335. Döring et al. (1984b) also

Figure 2.21. Sequences of double Ds and derivations of it in sh-m5933 (Döring et al., 1984b). "→" indicates the 11 bp-inverted repeats; "⇝" indicates 8 bp-direct repeats. Details are discussed in the text

(A) Sequence of double Ds with its termini and flanking nucleotides
(B) Double Ds structure. The numbers show the positions of the termini of Ds element
(C) One of the Ds hypothetical structures transposed from (B) structure
(D) Hypothetical structure of a revertant of sh-m5933 showing deletion of part of (C)


11bp
8bp
11bp
8bp
11bp
8bp
11bp
(A)
TAGGGATGAAA
CGAAGTGG
TAGGGATGAAA
CCACTTCG
TTTCATCCCTA
CGAAGTGG
TTTCATCCCTA
1
2
3
4
(B)
Ds at 3'junction of sh-m5933
(C)
Ds at 5'junction of sh-m5933
(D)
deletion
Ds at 5'junction in Sh-r5(revertant)

assumed that the 11 bp-inverted repeats may be sufficient for transposition by an Ac-encoded function of a DNA sequence bracketed by these inverted repeats, and more complicated structures can be built from the simple unit Ds. They found that the data obtained by Courage-Tebb et al. (1983) utilizing DNA blotting analysis of the inserted DNA adjacent to the 5' junction in sh-m5933 are compatible with their assumption (Figures 2.20 and 2.21). Hypothetical structures were proposed to explain the double Ds at the 3' junction of sh-m5933, the duplicated 5' junction fragment between the Sh and Ds sequences, and a revertant of sh-m5933, Sh-r5 by Döring et al. (1984b) (Figure 2.21). The double Ds at the 3' junction of sh-m5933 is given in Figure 2.21B. The sequence located at the 5' junction of Sh and Ds DNA sequence is an inverted copy of part of the double Ds at the 3' junction (Figure 2.21C). Part of the 2 kb sequence is present only once at the 5' junction while the other part is present twice. The structure at the 5' junction of the insertion consists of 1.5 copies of the 2 kb Ds. The deletion of part of the Ds structure in the revertant Sh-r5 and the restriction pattern of the nondeleted part are also compatible with the assumption that a unit Ds has been excised (Figure 2.21D). Only one terminus carries the 11 bp sequence in the remaining half Ds element. However, the chromosome breaks at this site are still detected. Döring et al. (1984b) assumed that these breaks are probably not dependent on the presence of the 11 bp sequence at both termini.

In addition, many direct and inverted repeats were found in this Ds sequence. Rearrangements involving these sequences are conceivable

and may provide a basis for "changes in state" of Ds (Döring et al., 1984b).

2.6.2.1.2. Ds at the Adh1 locus Sachs et al. (1983) sequenced a Ds insertion mutation of the Adh1 gene, Adh1-Fm335. They found an insertion of 405 bp in this Ds mutant between nucleotides 45 and 46 in the mRNA sequence. The results also showed that the insert is bounded by 11 bp repeats and to be AT-rich, flanked by direct 8 bp repeats which represents a duplication of base pairs 38-45 of the progenitor sequence (see Figure 2.6a and Figure 2.8a, b).

In the revertants of Adh1-Fm335, Sachs et al. (1983) found the Ds insertion had been excised clearly but that the direct 8 bp sequence which flanked the Ds insertion in the mutant was retained. However, there are changes of sequence at the new junction of the duplicated segments which has been mentioned before in section 2.2.3 (see Figure 2.6b, c, d, e).

Sachs et al. (1983) also supposed that the 11 bp termini may be the essential components of Ac-induced excision of the Ds element.

Döring et al. (1984a) reported that the Adh1-2F11 allele which was selected from the Ds mutant bz2-m was a Ds insertional mutation. The restriction map of the Ds in Adh1-2F11 showed the presence of a 1.3 kb Ds insertion in the Adh1 gene. All or part of this Ds insertion was transcribed.

The results of Sachs et al. (1983) and Döring et al. (1984a) indicated the different sizes of Ds elements in the Adh1 gene. This probably can not be a simple transposition of the same Ds from bz2-m to Adh1

because, in Southern experiments when the cloned Ds insertion from Adh1-Fm335 was used as a probe, Sachs et al. (1983) found approximately 40 homologous bands in the maize genome with this Ds probe. Teosinte also shows about the same number of the Ds-related sequences. Tripsacum also has approximately 20 sequences that are hybridized to this Ds probe. Additionally, Döring et al. (1984a) assumed that the Ds is an internal deletion of parts of Ac and that different isolates of Ds differ in deletion size (will be discussed in the next section, 2.6.2.1.3). Therefore, these deletions could be accounted for by the different sizes of Ds elements.

Sutton et al. (1984) reported on the molecular analysis of Ds at the Adh1 gene. They obtained the same results as Sachs et al. (1983). The results revealed that the 405 bp sequence is a Ds element flanked by 8 bp-direct repeats which is a duplication of the 8 bp existing at the point of insertion in the 5' untranslated region of the gene. The insertion sequence is AT-rich and has 11 bp-inverted-repeat sequences at its termini (see Figure 2.8b). In the revertants, the insertion with its inverted repeats is deleted, but the 8 bp-direct repeats remain in modified form (Figure 2.8c, d) which are exactly the same as the findings of Sachs et al. (1983).

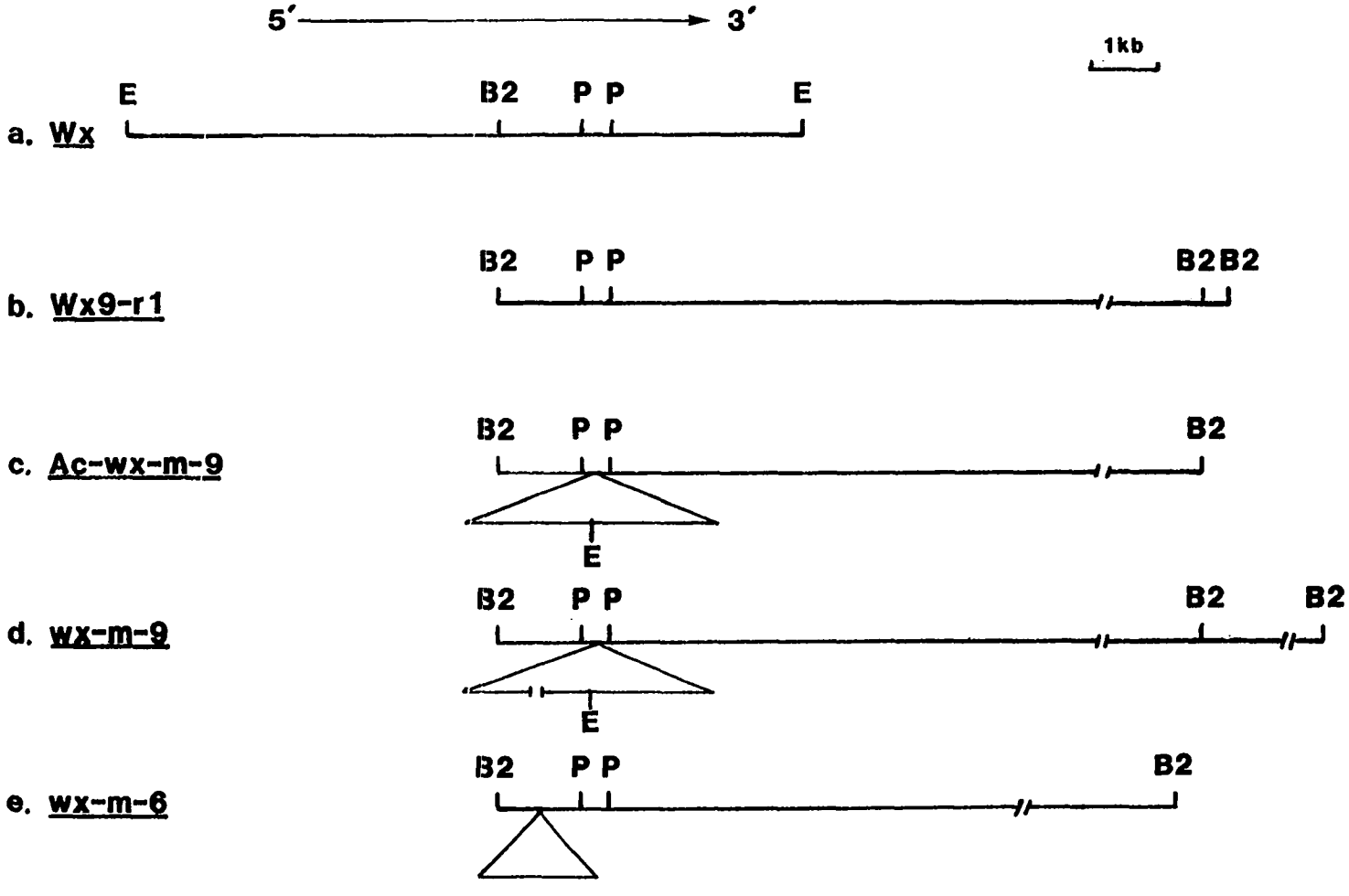
Sutton et al. (1984) also found at least 30 sequences in the maize genome related to the Ds element. This is compatible with the results of Sachs et al. (1983) and agrees with the assumption of Döring et al. (1984a) that explains the diversity of sizes of Ds elements at different loci.

2.6.2.1.3. Ds at the Wx locus Shure et al. (1983) isolated and identified the Wx locus and its gene product by using the Wx allele and three mutable alleles, wx-m-6, wx-m-9, and wx-m-8. wx-m-6 and wx-m-9 alleles have Ds elements inserted at the Wx locus, while the wx-m-8 allele is attributable to the insertion of the I element of Spm(En) controlling element system. Using wx-m-6, they found a 2.4 kb insertion of the Wx locus caused the mutation. This 2.4 kb insertion is the Ds element.

In a subsequent study, Fedoroff et al. (1983b) isolated the Ac and Ds elements from Ac-wx-m-9 (Ac insertional mutation of Wx), wx-m-9 and wx-m-6 (Ds insertional mutations). Evidence of the restriction endonuclease fragments containing part of the Wx locus with Ac or Ds insertion is presented that the genetically defined Ac element is a 4.3 kb insertion at the Wx locus in a strain homozygous for the Ac-wx-m-9 allele. The heteroduplexes between the Wx Bgl II fragments cloned from Ac-wx-m-9 and its revertant (Wx 9-r1) showed homology except for a 4.5 kb region near one end. The heteroduplexes between the Ac-wx-m-9 Bgl II fragment and a cloned Wx EcoR1 fragment (Shure et al., 1983) also differed by a 4 kb sequence within the region of overlap between them. Thus, the heteroduplex and restriction map confirm the Ac insertion and its location within a 0.23 kb Pst1 fragment near the 3' end of the mRNA coding sequence (Figure 2.22a, b, c). Restriction analyses revealed that the Ds element in the wx-m-9 is almost identical to the Ac element at precisely the same site within the Wx transcription unit. The 4.1 kb Ds insertion lies within the same Pst1 fragment within which the Ac element is inserted in the Ac-wx-m-9. However, the cloned

Figure 2.22. The structure of EcoR1 and Bgl II fragments of the Wx locus (modified from Fedoroff et al., 1983b). Details are discussed in the text

B2 = Bgl II
E = EcoR1
P = Pst1


5′
3′
1kb
E
B2
P P
E
a. Wx
B2
P P
B2 B2
b. Wx9-r1
B2
P P
B2
c. Ac-wx-m-9
E
B2
P P
B2
B2
d. wx-m-9
E
B2
P P
B2
e. wx-m-6

PstI fragments differ from each other by the absence from wx-m-9 of a sequence of less than 0.2 kb present near the center of the Ac element in Ac-wx-m-9 (Figure 2.22d). The structure of the wx-m-6 transcription unit differs from that of the progenitor Wx allele by the presence of an insertion in a 1 kb PstI fragment near the 3' end. This insertion proved to be 2.0 kb in length in wx-m-6 and is entirely homologous to a 1 kb sequence at each end of the Ac element in Ac-wx-m-9 (Figure 2.22e). The evidence of the restriction maps show that the Ds element in wx-m-6 is also related to Ac in Ac-wx-m-9. The Ds element in wx-m-6 is inserted in the opposite orientation to the Ac in Ac-wx-m-9 and to the Ds in wx-m-9 at a different site in the transcription region.

It is conceivable that Ds is a complementable mutant of Ac since the Ds element can transpose and cause chromosome breaks only in the presence of Ac, while Ac can fulfill both functions autonomously. More convincingly, some Ds elements have been derived from Ac elements (for example, bz-m-2 (McClintock, 1956c)). The findings of Fedoroff et al. (1983b) could explain these findings of Döring et al. (1984a).

2.6.2.2. Spm(En) system

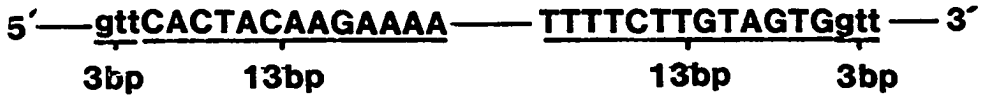
2.6.2.2.1. Receptor element at the Wx locus Schwarz-Sommer et al. (1984) cloned the Wx locus from strains carrying the wild type and wx-m-8 alleles. They obtained a 2 kb insertion within the transcribed region of the Wx gene in wx-m-8. This insertion proved to be the receptor element (I) of Spm(En) system.

Schwarz-Sommer et al. (1984) sequenced the I element which consists of 13 bp-inverted repeats at its termini flanked by 3 bp-inverted repeats

(Figure 2.23). This is different from the discovery of the Ds element and also different from Cin 1 consisting of 5 bp-terminal-inverted repeats flanking 5 bp repeats.

Figure 2.23. Sequences of the terminal 13 bp and its flanking 3 bp of I element of wx-m-8 allele (Schwarz-Sommer et al., 1984)

I of wx-m-8



3. MATERIALS AND METHODS

3.1. Gene Symbols and Definitions of Terms

Alleles or elements	Description or phenotype
A	A dominant allele of one of the genes necessary for the synthesis of anthocyanin in the aleurone (chromosome 3).
a	A recessive allele of A, colorless aleurone, not responsive to any known regulatory element.
a-m(r)	A recessive allele of A, responds to En. In the absence of En, the aleurone is colorless; in its presence, colored spots are produced on a colorless background.
a-m-1	A recessive allele of A, responds to En. In the absence of En, the aleurone is pigmented pale; in its presence, colored spots are produced on a colorless background.
a-m-1(5720)	One state of a-m-1, responds to En. In the absence of En, the aleurone is light pale; in its presence colored spots are produced on a colorless background.
ax-1	An allele of A on chromosome 3 at 3L.75, causes a genetic alteration that includes the loss of A and Sh2 and a gene controlling chlorophyll synthesis. A haploviable deficiency with normal transmission

Alleles or elements	Description or phenotype
	through the female and variably reduced transmission through male (Natarajan, 1981).
Sh2	Nonshrunken endosperm, 0.25 map units from A. Abbreviated to Sh in this study.
sh2	Shrunken endosperm, a recessive allele of Sh2. Abbreviated to sh in this study.
Et	A dominant allele on chromosome 3, 12 map units distal to A. Necessary for the expression of a smooth surface on the kernel.
et	Etched, etched marking on the aleurone surface. A recessive allele of Et. Homozygous et genotype yields virescent leaves in early seedling stage and turns to green as seedling grows.
C	A dominant allele of one of the genes necessary for the synthesis of anthocyanin in the aleurone (chromosome 9).
c	A stable recessive allele of C, colorless aleurone.
Sh1	A dominant allele, nonshrunken endosperm (chromosome 9).
sh1	A recessive allele of Sh1, shrunken endosperm.
sh-m	A recessive allele of Sh1 due to a Ds in its vicinity and response to Ac. In the absence of Ac, the endosperm is shrunken; in its presence, nonshrunken sectors are produced (sh1 → Sh1) on the shrunken endosperm background.

Alleles or elements	Description or phenotype
Wx	Starchy endosperm, a dominant allele on chromosome 9. Pollen stains purple with IKI.
wx	Waxy endosperm, a recessive allele of Wx. Pollen stains red with IKI.
En	Enhancer, a regulatory element acting in trans, necessary for mutability of En responsive alleles.
Autonomous En	En residing at a gene locus, controlling the mutability of that gene. It is inseparable from the locus by crossover.
Ac	Activator, a regulatory element necessary for mutability of Ac responsive alleles. With some states of Ds, causes chromosome breakage at the site of Ds.
Ds	Dissociation, a receptor element. In combination with Ac, some states result in chromosome breakage at the site of Ds.

Terms

Controlling elements	A collective term for transposable elements associated with mutable alleles that affect gene activity.
Controlling element system	A system includes two units, receptor and regulatory elements. They express a specific interaction.
Receptor elements	Elements such as dt, Ds, and I that suppress gene activity when in cis position to the locus.

Terms	Description or phenotype
Regulatory elements	Elements such as Dt, Ac, and En which alter or excise receptor elements so that the locus under control becomes functional.
Autonomous control (of a-m)	Represents the control of mutability of the a-m mutable allele by a cis-acting En, closely associated with the a locus. In such cases, the mutability is inseparable from the locus by crossover.
Mutability	Variegated phenotypic expression in a tissue that is characterized by more than one phenotype such as colored spots or shrunken sectors on a colorless background.
Patterns	Mutability patterns expressing mutation events. Different patterns express differences in timing and frequency of mutability events.
State	Refers to an allelic state that is expressed as a definable mutable pattern, ex. a state of En refers to a pattern expression of that En.
Transposition	Movement of a controlling element from one position in the genome to another.
Testcross	Cross between a heterozygous genotype and a tester that tests that genotype. The tester is limited to a-m-1 sh/a-m-1 sh in this study. In all crosses, the a-m-1 sh/a-m-1 sh parents are used as a female.

Terms	Description or phenotype
Sibcross	Represents a cross of the individuals derived from a single ear that is obtained in the progeny of a cross.
Testers	
a-m-1 sh	Line homozygous for a-m-1, A2, C, C2, and R with homozygous sh allele.
a sh	Homozygous line for a, A2, C, C2, and R with homozygous sh allele.
a et	Line homozygous for a, A2, C, C2, and R with homozygous et and Sh.
C Ds sh-m	Homozygous line for C, Ds, and sh-m and A, A2, C2, and R.
c sh wx	Line homozygous for A, A2, c, C2, R, sh1, and wx.

3.2. Source of Material

A mutable allele, a-m (dense), at the a locus on chromosome 3 expressing a dense mutable pattern (fine dense colored spots on colorless background) originated from an A allele in a pale green mutable stock. The mutability of the original a-m (dense) is autonomously controlled (En is an insertional mutation of a locus) (Peterson, 1961). From a testcross of this a-m allele with a sh/a sh tester used as male, numerous exceptional pattern types arose [a-m(r), a-m(nr) (Peterson, 1961), a-m(papu) (Peterson, 1970), a-m(Au) (Peterson, 1978), a-m(pm) (Nowick and Peterson, 1980)].

When a plant having the genotype, a-m (dense)En Sh/a sh, was crossed onto a-m-1 sh tester in 1965, colored shrunken sectors were observed on the kernel with a fine spotting pattern (Figure 3.1). The pedigree of this exceptional derivative was traced back to the 1952 370-1 line and this derivative was developed from the common source 1965 1872 and 1873 series (Table 3.1). The colored shrunken sector illustrates a multiple gene loss event. Since this loss event is unique, the En in this allele, a-m 61138-3 (=a-m En) was designated as En61138-3 (Huang and Peterson, 1983). This exceptional derivative arising from the original a-m (dense) was used in this study.

3.3. Genetic Characterization of the Loss Events

3.3.1. Crosses

In an attempt to ascertain the genetic characteristics of the allele and to determine the extent of the loss events, the following crosses were made.

3.3.1.1. Testcross Plants containing the En61138-3 allele were crossed as males onto plants having the a-m-1 sh genotype. The reason for this procedure was to reveal the shrunken sectors that are easy to expose on the sh gene background if En61138-3 is lost. Data are collected on the loss events from the testcross progeny.

3.3.1.2. A test for the presence of Ac The C Ds sh-m stock and a c sh wx tester were used in this experiment to assess the loss event induced by En61138-3.

3.3.1.3. Other crosses The following crosses were made to determine the extent of the loss events on chromosome 3.

Figure 3.1. A kernel showing the colored shrunken sectors (→) arising from a cross of a-m-1 sh/a-m-1 sh x a-m En Sh/a-m-1 sh

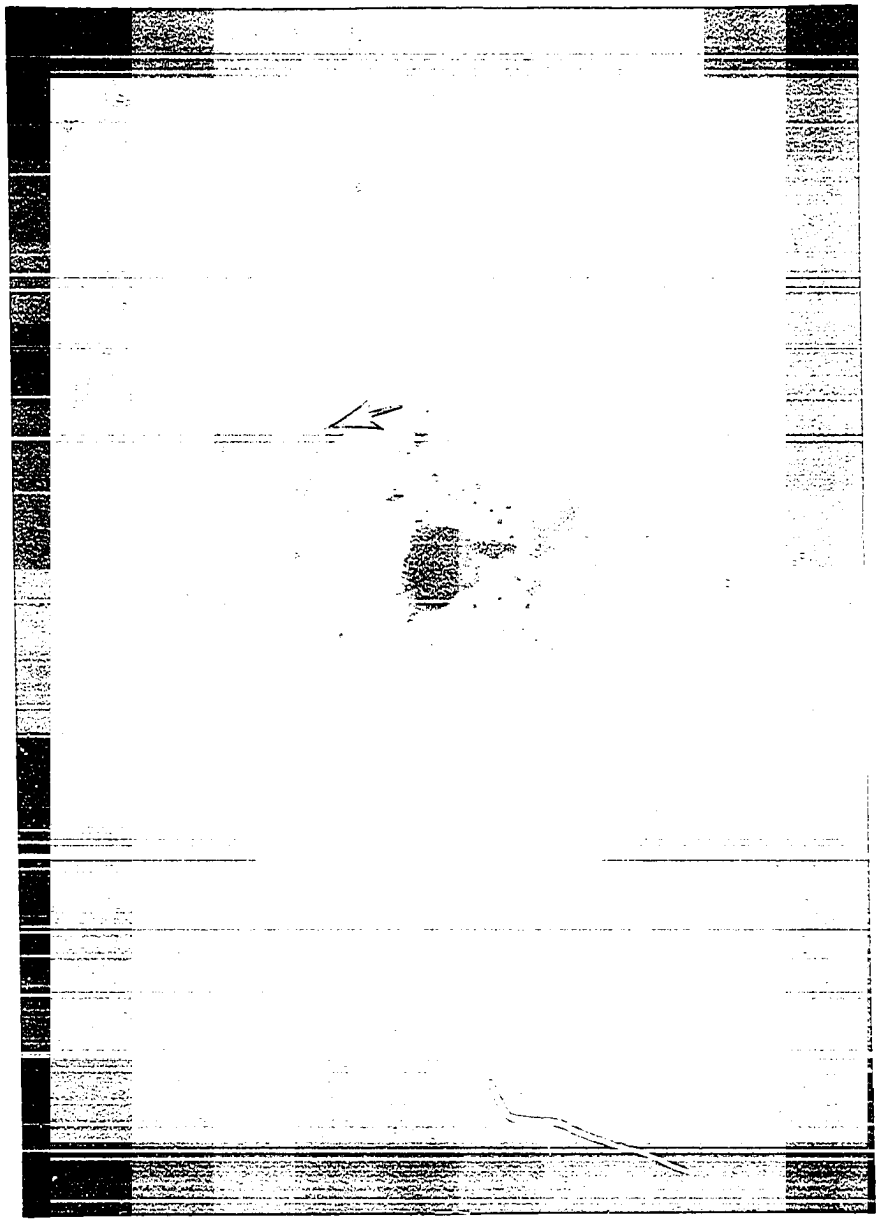


Table 3.1. Pedigree of the exceptional derivative with colored shrunken sectors arising from a-m-1 sh/a-m-1 sh x a-m En Sh/a sh (1965 1872 and 1873 series). The a-m (dense) (=a-m En) allele was maintained by crossing with a sh/a sh males as illustrated in this table

Year	Ear number	Phenotype	Genotype of crosses
1980	1857,1858	Fine spotting with colored shrunken sectors	a-m-1 sh/a-m-1 sh x a-m En Sh/a-m-1 sh (En61138-3)
1965	1872,1873	"	a-m-1 sh/a-m-1 sh x a-m En Sh/a sh
1964	300	dense	a-m En Sh/a sh x a sh/a sh
1963	161-2	"	a-m En Sh/a sh x a sh/a sh
1962	29-4	"	a-m En Sh/a sh x a sh/a sh
1961	138-3	"	a-m En Sh/a sh x a sh/a sh
1960	644-1	dense	a-m En Sh/a sh x a sh/a sh
1957	222-9	f. cl. hi.[a]	a-m En Sh/a sh x a sh/a sh
1956	14-3	"	a-m En Sh/a sh x a sh/a sh
1953	37-21	"	a-m En Sh/a sh x a sh/a sh (a-m (dense) = a-m En arose)
1952	370-1[b]	"	a Sh et/a Sh et x a-m En Sh/a-dt Sh

[a] f. cl. hi. = fine, clear, heavy.

[b] Original a-m containing plant (Peterson, 1953b, 1956). See Peterson (1961) for the array of mutation patterns.

A. Test on ax-1 and a sh alleles.

ax-1/a-m-1 sh, ax-1/a et, and a sh/a sh testers were crossed by plants having the genotype of En61183-3/a-m-1 sh (=a-m En Sh/ a-m-1 sh). If, for instance, the a-m En Sh segment is lost, the colorless shrunken sectors should appear on ax-1 and a sh background. Therefore, these crosses will expose the extent of the loss event. In addition, the ax-1/a-m-1 sh and ax-1/a et parents used as males were crossed onto a et and a-m-1 sh testers, respectively, to confirm the presence of the ax-1 allele in the female parents crossed by the En61138-3 allele.

B. Test on a-m-1(5720) allele.

The a-m-1(5720) Sh/a sh genotype crossed by En61138-3/a-m-1 sh to assure that the a is lost concomitantly with the loss of En and Sh. The expected phenotypes are pale sectors appearing on round spotted kernels and colorless shrunken sectors appearing on round spotted kernels.

C. Test on a et allele.

Because et homozygotes or hemizygotes show virescent color on the leaves in the seedling stage, this cross provides a good genetic marker for detecting the extent of the loss event induced by En61138-3 on chromosome 3.

Seedling progeny of the crosses of a et/a et used as female by En61138-3/a-m-1 sh males were examined in the greenhouse. The purpose is to search for virescent sectors (stripes) on the green seedling leaves resulting from the loss of Et from

En61138-3. This will give evidence that the segment from a up to the end of the long arm of chromosome 3 is lost.

3.3.2. Sectoring pattern determination

Different sizes and frequencies of colored shrunken sectors appear on the kernel in the testcross progeny. To differentiate these sectoring patterns, sector size larger than 4.0 mm^2 is assigned as large sector (ℓ); sector size less than 0.25 mm^2 is classified as small sector (s); and the size between 4.0 mm^2 and 0.25 mm^2 is medium (m). However, only two of the sector sizes were assigned in 1980; large size (>0.5 mm^2) and small size (<0.5 mm^2). All the sector sizes and frequencies were determined under the stereoscopic microscope. The size was measured with an ocular micrometer.

Different patterns of the loss of mutability are shown in Figure 3.2. The ℓ, m, s letters represent the sizes of the sector. The numbers represent the frequency of the sectors on the kernel. These patterns are designated as states of En61138-3 in this study.

3.3.3. Determination of the frequency for the loss event

Frequency of loss events = $a \times \frac{b}{c}$

a: Mean frequency of kernels with loss sectors per ear in a population.

b: Average number of cells per colored shrunken sector on a kernel.

c: Total number of aleurone cells of a kernel. 160,000 cells were estimated by Stadler (1944).

Three populations were available for the estimation of the frequency

of loss events. The random samples of the kernels expressing the loss phenomena were collected from the selfed populations of 1982 and 1983, and the testcross population of 1983.

The number of cells in each sector was estimated by using an ocular micrometer with grid in a stereoscopic microscope. The number of cells in each grid was measured prior to counting the total number of cells in a sector.

The total number cells of the aleurone was estimated by Stadler (1944). Approximately 160,000 cells were measured in the aleurone of a kernel in maize.

3.3.4. Distribution of the loss events

In an attempt to understand the properties of the loss event, Poisson distribution was tested in six families of 1981. Since the occurrence of the loss of mutability is a rare event, the fitness test of the Poisson distribution will show the property of the distribution of the sectors in a limited time or space interval.

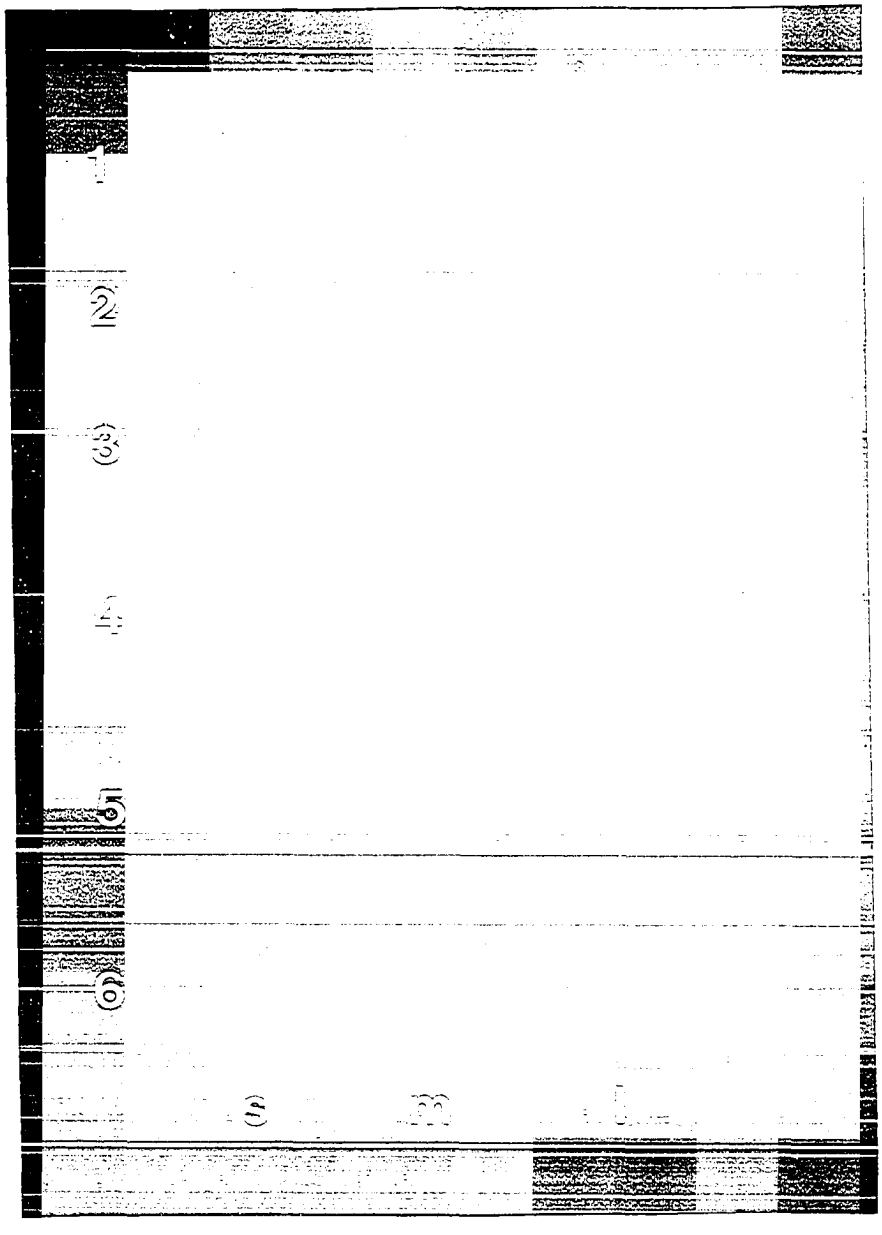
3.4. Heritability of the Level of the Loss Events

3.4.1. On a family basis

The round spotted kernels on the colorless background with and without loss sectors were selected from six parental ears derived from testcrosses of 1980. The plants derived from these kernels were test-crossed onto a-m-1 sh tester. The six parental ears expressed different levels of the loss of mutability, ranging from 28.2% to 1.7%. The parent with the lowest frequency of the loss of mutability was bulked

Figure 3.2. Sectoring patterns of the loss event of En61138-3

ℓ = large size; m = medium size; s = small size
1 = one sector
2 = two sectors
3 = three sectors
4 = four sectors
5 = five sectors
6 = more than 5 sectors



as a sample from the ears showing low frequency of the loss event. The frequency of the loss event of this parent was estimated by weighting the frequencies of the loss events of the ears, the components of that parent. The frequency of the parent with large sectors derived from a bulked sample of the kernels showing large sectors on different ears was also a weighted value. The frequencies of the loss event of these six parents are shown in Table 4.13 to Table 4.18. The weighted frequency was calculated as follows:

$$\text{weighted frequency} = \frac{\Sigma fiXi}{\Sigma fi}$$

fi: No. of kernels of i^{th} ear in the bulked sample.

Xi: Frequency of the loss event of i^{th} ear.

The parent-offspring correlation representing the heritability of the En61138-3 was calculated from the data of the parents and their corresponding testcross progeny.

Similar procedures were used for nine parents and their corresponding testcross progeny in 1982 to confirm the heritability of the En61138-3. The range of the frequencies of the loss event is from 16.2% to 2.4% (see Tables 4.19 to 4.27 and Figure 4.12).

3.4.2. Heritability of the states of En61138-3

3.4.2.1. On a family basis The same data obtained from five families in 1981 (Tables 4.13 to 4.18, except Table 4.14), nine families in 1982 (Tables 4.19 to 4.27) and the data of 1983 testcross progeny (not shown) were used in parent-offspring correlation analysis to show the heritability of the states of En61138-3 on a family basis.

3.4.2.2. Relations between the frequency of loss events and the timing of loss events Parent-offspring correlations were calculated between the total frequency of the loss event in the parent and that of the different sizes of sectors in the progeny. The data of 1981, 1982, and 1983 were used for this kind of analysis. Only the data of 1982 were used for testing the closeness of relation between the frequency of loss events and the timing of loss events in the same generation.

3.4.2.3. Correlation between the sizes of the sectors and their frequencies Correlation between the sizes of the sectors and their frequencies was calculated by using the data from the testcross progeny in 1983.

3.4.2.4. Comparison of the states of En61138-3 derived from the kernels with and without colored shrunken sectors Data from nine families of 1982 and 12 families of 1983 were analyzed to compare the differences in the frequencies of different sizes of sectors on the kernels derived from the testcrosses in which the male parents (a-m En Sh/ a-m-1 sh) were from the kernels with and without colored shrunken sectors respectively.

3.4.2.5. Single kernel basis The parents were selected with a specific state of En61138-3 according to the designated sectoring patterns of loss event (Materials and Methods, section 3.3.2). The states of En61138-3 on the kernels from the testcross progeny of these parents were recorded. The average percentage of the same state as its parent in the testcross progeny represents the heritability of the states of En61138-3 on single kernel basis. Three parental kernels

expressing "s-1" state, two parental kernels with ℓ or "ℓ-1" state, and four with "zero" state were selected for this experiment.

3.5. Factors Affecting the Occurrence of the Loss Events

3.5.1. Kinds of crosses

The following tests were made to determine the effect of different kinds of crosses on the level of En61138-3 loss events.

A. Compare the effect of testcross, selfing, and sibcross on the level of En61138-3 loss events.

B. Test the effects of female and male parents derived from plants with high and low level of En61138-3 loss events and their continuity on the frequency of the loss events.

C. Test the possibility of the modifiers in testcross, selfing, and sibcross propulations.

All comparisons were tested by t-test or χ^2-test.

3.5.2. Effect of main stalk and tiller of the same plant on the level of En61138-3 loss events

A. Two families were selected in 1982, derived from the parental ears A and B (see Table 4.19 and Table 4.20). Both of them express a high frequency of loss events. Crosses of the a-m-1 sh tester and the main stalk and the tiller of the same plant used as males of these two families were made in 1982. At the same time, the main stalk and the tiller ears were selfed. Compare the level of En61138-3 loss events derived from the main stalk and tiller by analysis of variance and contingency χ^2-test.

B. Five families derived from the parental tiller ears showing different levels of En61138-3 loss events ranging from 19.4% to 0.91% were selected in 1983. A similar procedure with that in 1982 was followed to test the difference of the level of the En61138-3 loss events between main stalk and tiller.

C. Heritability of the En611383-3 loss events on main stalk and tiller through parental main stalk and tiller to their corresponding progeny main stalk and tiller respectively was calculated by parent-offspring correlation analysis and contingency χ^2-test. Also, the heritability of the exceptional expression of the En61138-3 loss events in main stalk or tiller was tested by contingency χ^2-test.

D. Comparison of the states of En61138-3 in main stalk and tiller in 1982 and 1983 by t-test.

3.5.3. Effect of planting date on the level of En61138-3 loss events

Three families derived from parental ears with different levels of the loss events were planted on May 8 and May 24, 1982. Two parental ears show high levels of En61138-3 loss events with 38.2% and 12.8%, respectively. The third one bulking from the ears with a low frequency of En61138-3 loss events shows 1.04% of the loss frequency (weighted frequency).

All the individuals of these three families were crossed onto an a-m-1 sh tester. A comparison was made of the frequency of the En61138-3 loss events between the two planting dates by an analysis of variance.

3.6. Response to Selection on the Loss Events

Selected individual plants with high and low frequencies of the loss events, respectively, from each population derived from the plant with high or low frequency of loss events through three years. This bidirectional scheme is shown in Figure 4.29 to Figure 4.33.

Compared the responses to selection on the En61138-3 loss events and also measured the heritability of the En61138-3 loss events in this selection program. All the data are obtained from the testcross progeny in this selection experiment. The heritability was measured by parent-offspring correlation and χ^2-test.

3.7. Analyses of the Data

Appropriate statistical methods used in this study are as follows.

3.7.1. Simple linear correlation (Steel and Torrie, 1980; Le Clerg et al., 1962)

The correlation coefficient measures a co-relation between two variables and tells us something about a joint relationship between these two variables. The simple correlation coefficient is used to represent the heritability of the frequency of the loss events induced by En61138-3. In fact, this coefficient is identical to the standard unit heritability (Frey and Horner, 1957). The formula of the correlation is:

$$r = \frac{\Sigma(x-\bar{x})(y-\bar{y})}{\sqrt{\Sigma(x-\bar{x})^2 \ (y-\bar{y})^2}}$$

where x,y are the random pairs of observations. y values are obtained from several populations, each population being determined by a

corresponding x value. x and y represent the frequency of the loss event of parents and that of their corresponding progeny respectively.

3.7.2. t-test (Steel and Torrie, 1980)

A. Comparison of two sample means, independent samples and equal variance.

$$t = \frac{(\bar{y}_1-u_1)-(\bar{y}_2-u_2)}{S_{\bar{y}_1-\bar{y}_2}} = \frac{\bar{y}_1-\bar{y}_2}{S_{\bar{y}_1-\bar{y}_2}}$$

which for Ho: $u_1=u_2$ and

$$S_{\bar{y}_1-\bar{y}_2} = \sqrt{S^2(\frac{1}{n1} + \frac{1}{n2})}$$

where

$$S^2 = \frac{(n_1-1)S_1^2 + (n_2-1)S_2^2}{(n_1-1)+(n_2-1)}$$

n_1 and n_2 are the sample sizes for sample 1 and sample 2, respectively. S_1^2 and S_2^2 are the sample variances for sample 1 and sample 2, respectively.

B. Comparison of sample means; meaningfully paired observation:

$$t = \frac{\bar{D}}{S_{\bar{D}}}$$

$$D = \Sigma(Y_1-Y_2)$$

$$\bar{D} = \frac{D}{n}$$

$$S_D^2 = \frac{\Sigma D^2-(\Sigma D)^2/n}{n-1}$$

$$S_{\bar{D}} = \sqrt{\frac{S_D^2}{n}}$$

where

Y_1, Y_2: paired observations in a sample;

D: the difference between paired values;

$\bar{D}$: mean of D; and

n: the number of pairs.

3.7.3. χ^2-tests (Steel and Torrie, 1980; LeClerg et al., 1962)

A. Goodness of fit tests:

The term "goodness of fit" refers to the comparison between some observed sample distribution and theoretical frequency distribution. The χ^2-test for goodness of fit can be applied to data grouped into classes where it is desired to compare them with a theoretical ratio. The general formula is as follows:

$$\chi^2 = \sum_i \frac{(o_i - e_i)^2}{e_i}$$

o_i: i^{th} observed value; and

e_i: i^{th} expected value when the hypothesis is true, that is, the theoretical value.

B. Contingency χ^2:

When observations have been classified with respect to two or more attributes, it may be desirable to determine whether or not the two (or more) attributes are associated. The χ^2-test is used to test the hypothesis that the two attributes are independent. The computation is as follows:

(1) Contingency χ^2-test for a 2x2 table, for instance:

	No. of kernels without loss events	No. of kernels with loss events
Parent	a	b
Progeny	c	d

(2) The corrected χ^2 formula for this 2x2 table, because there is only one degree of freedom in a 2x2 table, is as follows:

$$\chi^2(\text{corrected}) = \frac{n(|ad-bc|-n/2)^2}{(a+b)(a+c)(c+d)(d+b)}$$

where n = a+b+c+d.

3.7.4. Poisson distribution

A. Poisson distribution:

The number of clicks produced by a Gieger counter in a unit of time, the number of yeast cells per cubic millimeter in a suspension, and insect counts in field plots are the examples of situations involving a certain kind of isolated event in a time or space continuum. The random sampling in these examples may yield data which follow a Poisson distribution.

Probabilities for a Poisson distribution are given by:

$$1 = e^{-\lambda} + e^{-\lambda}\lambda + \frac{e^{-\lambda}\lambda^2}{2!} + \frac{e^{-\lambda}\lambda^3}{3!} + \frac{e^{-\lambda}\lambda^4}{4!} + \ldots$$

which describes the occurrence of an isolated event in a time or a space continuum. It should be noted that e=2.718, the base of natural logarithms. λ is used to represent the observed mean occurrence of the event. $e^{-\lambda}$ represents the probability of zero

events, $e^{-\lambda}\lambda$ the probability of one event, $\frac{e^{-\lambda}\lambda^2}{2!}$ the probability of two events, and so forth.

The expected frequency of the isolated events may be calculated from this Poisson distribution by using the observed values.

B. Test of randomness by χ^2 (test for goodness of fit):

χ^2-test can be used to determine the probability that the difference between the observed frequencies and the expected frequencies of the isolated event occurred by chance alone. In other words, this test can be used to determine whether an event is distributed randomly or has a tendency toward clumping in a Poisson distribution. For instance, a bacteriologist may wish to know if bacterial growth on plates is random, or if unusual growth has occurred on certain plates. This kind of problem may be approached by this χ^2-test shown in the following:

$$\chi^2 = \sum_i \frac{(o_i - e_i)^2}{e_i}$$

where o_i: i^{th} observed value, and e_i: i^{th} expected value.

3.7.5. Analysis of variance

Appropriate analysis of variance was used when it was required in the experiment.

The Statistical Analysis System (SAS) programs were used to analyze most of the data in this study with the computer of the Computer Center of Iowa State University.

4. RESULTS

4.1. Genetic Characteristics of En61138-3

4.1.1. Characterization of the loss phenomenon

A mutable allele, a-m (dense), showing very dense spots in the kernels on colorless background, arose in a pale green mutable stock. En is associated with the a locus of this mutable allele (a-m En) (Peterson, 1961). An exceptional derivative arose from the crosses of the a-m-1 sh tester and the plants containing the a-m (dense) allele [a-m-1 sh/a-m-1 sh x a-m En Sh/a sh (Cross 4.1.1A) (see Table 3.1, crosses of 1965)]. This exceptional derivative shows a fine spotting pattern and, in addition, displays colored shrunken sectors on the round spotted kernels when heterozygous with a-m-1 sh. The genotype of these kernels with colored shrunken sectors must be a-m En Sh/a-m-1 sh. These colored shrunken sectors illustrate a multiple gene loss event. Shrunken sectors are indicative of loss of the Sh on the sh gene background. The appearance of color pigmentation within the shrunken sectors shows that the A function and En are also lost simultaneously with the Sh gene on chromosome 3. Either a simultaneous loss of En and Sh or a simultaneous loss of En, Sh, and function of A could yield color pigmentation with shrunken phenotype on a-m-1 sh background. This gives rise to a question of the extent of the multiple gene loss event which will be discussed in detail in the following paragraphs and sections.

The allele showing the multiple gene loss is designated as a-m61138-3 (Materials and Methods). In an attempt to characterize this loss event and to determine the extent of the loss event, plants

containing the a-m61138-3 allele derived from the exceptional kernels with colored shrunken sectors of Cross 4.1.1A were crossed as males onto the receptive a-m-1 allele that is linked to sh. This is because the colored shrunken sectors can readily be epxressed with a sh gene background. Two kinds of this cross were carried out as follows:

♀	♂	
a-m-1 sh/a-m-1 sh	x a-m En Sh/a-m-1 sh	Cross 4.1.1B
a-m(r) Sh/a-m-1 sh	x a-m En Sh/a-m-1 sh	Cross 4.1.1C

From these crosses, kernels with colored shrunken sectors were found among the progeny (Figure 4.1). The results of several crosses are presented in Table 4.1. The progeny from these crosses showed that

(1) The size and the frequency of these colored shrunken sectors varied on different kernels (Figure 4.1).

(2) The percentage of kernels with colored shrunken sectors on individual ears varied among ears.

(3) All the round kernels were spotted with a colorless background in Cross 4.1.1B.

(4) Two thirds of the round kernels were spotted on a colorless background in Cross 4.1.1C.

(5) All of the shrunken kernels were colored in both Cross 4.1.1B and Cross 4.1.1C.

The results of (3), (4), and (5) indicate that there is no independently segregating En and it is most probably located at or in a very close proximity to the a-m locus.

Figure 4.1. The loss events induced by En61138-3
(A) An ear illustrating the loss event that arose from a-m-l sh/a-m-l sh x a-m En Sh/a-m-l sh (Cross 4.1.1B)
(B) Three kernels expressing different sizes of loss event: (1) large sector, (2) medium sector, (3) small sector. The loss sectors are indicated by an arrow (→)

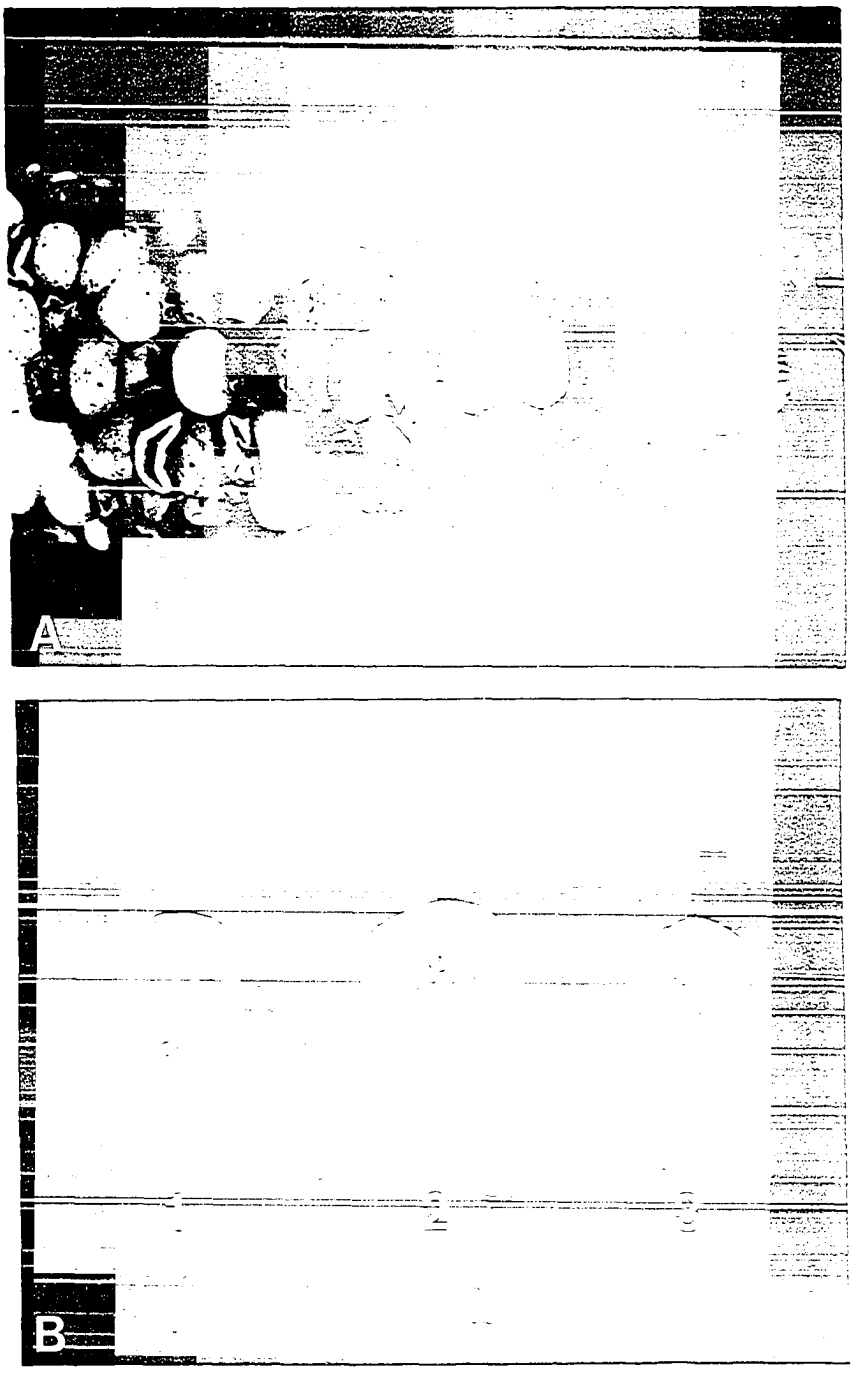

A
B

Table 4.1. Phenotypic frequencies from crosses of a-m-1 sh/a-m-1 sh or a-m(r) Sh/a-m-1 sh ♀♀ by ♂♂ plants originating from round kernels with spots on a colorless background with colored shrunken sectors (a-m En Sh/a-m-1 sh) (Cross 4.1.1.B and Cross 4.1.1.C)

Crosses (1980)	Round						Shrunken	χ^2-test 1:1 (Crosses 4.1.1.B) 1:2:1 (Crosses 4.1.1.C)
	Nonspotted		Spotted			%	Non-	
	Colored	Color-less	No sector	Large[a] sector	Small[a] sector	with sector	spotted colored	
4.1.1B a-m-1 sh/a-m-1 sh x a-m En Sh/a-m-1 sh								
1. 2534/1857-2	1		21	1	3	16.0	24	ns[b]
2. 2550/1857-2t			82	2	0	2.4	99	ns
3. 0125/1857-3			190	0	5	2.6	184	ns
4. 0125/1857-4			153	2	10	7.3	198	ns
5. 0125/1857-5			89	1	9	10.1	95	ns
6. 2535/1857-6			158	0	26	14.1	216	ns
7. 2536/1857-7t	1		161	0	16	9.0	161	ns
8. 0125/1857-9			127	5	45	28.2	190	ns
9. 0125/1857-9			207	0	20	8.8	189	ns
10. 0125/1857-9t			186	2	12	7.0	186	ns
11. 2550-1/1857-10t			82	2	0	2.4	99	ns
12. 0104/1858-1m			69	0	1	1.4	64	ns

[a]The area of large sector is larger than 0.5 mm^2. The area of small sector is smaller than 0.5 mm^2.

[b]ns = Nonsignificant.

Table 4.1. Continued

Crosses (1980)	Round: Nonspotted Colored	Round: Nonspotted Colorless	Round: Spotted No sector	Round: Spotted Large[a] sector	Round: Spotted Small[a] sector	% with sector	Shrunken Non-spotted colored	χ^2-test 1:1 (Crosses 4.1.1.B) 1:2:1 (Crosses 4.1.1.C)
4.1.1C a-m(r) Sh/a-m-1 sh x a-m En Sh/a-m-1 sh								
13. 0719/1857-3	90	1	161	1	0	0.6	84	ns
14. 0948/1857-4	120		233	2	2	2.1	84	*
15. 2131/1857-7	54	1	93	0	1	1/1	45	ns
16. 4201/1857-7t	20		48	0	1	2.0	24	ns
17. 2132/1857-11	95		201	0	2	0.1	98	ns
18. 0942/1858A-1t	69		135	2	13	10.0	67	ns
19. 0944/1858-1t	20		68	0	1	1.4	24	ns

*Significant at the 5% level.

(6) All colored shrunken sectors are on round spotted kernels.

(7) Shrunken sectors appeared on round spotted kernels indicating that the Sh gene is lost on a sh gene background in both Cross 4.1.1B and Cross 4.1.1C.

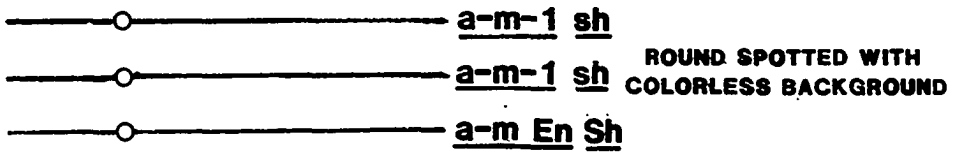
(8) The change from colorless to dark pale pigmentation coincident with the shrunken sectors shows that the En is lost simultaneously with Sh. The pale pigmentation of the a-m-1 allele is expected in the absence of En. The colored spots with colorless background might result from the response of a-m-1 to En. But with the loss of En and Sh, a-m will be expected to revert to A, and in such a situation colored shrunken sectors will also appear on spotted round kernels (Figure 4.2).

However, it is difficult to differentiate the color of shrunken sectors induced by A or by a-m-1. Therefore, from these observations of (6), (7), and (8), the extent of the loss event includes at least the A function, the controller of a locus mutability, En, and the Sh locus on chromosome 3. More evidence confirming this multiple gene loss at this a-m61138-3 allele including the A function, the En element and the Sh gene will be given in later sections.

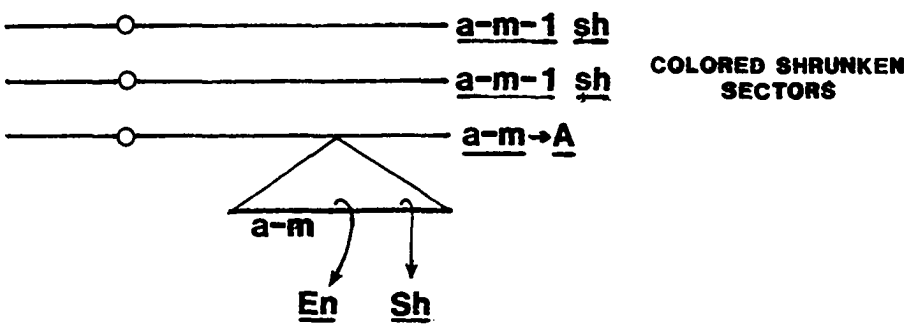
From these results, it has been established that the a-m61138-3 allele is autonomously mutable with En. Since this is a unique En, it will be identified as En61138-3 in all the following sections.

In further discussions, reference to the a En Sh loss, it can be considered that the loss includes the A function, the En element, and the Sh gene.

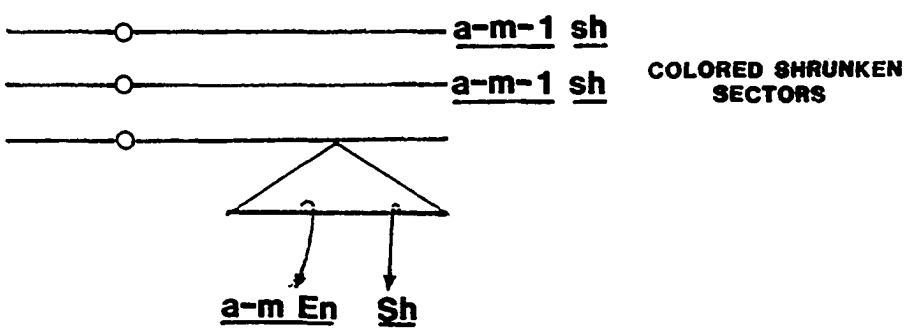
Figure 4.2. Diagram showing the cases of the occurrence of the loss events in the endosperm cells having the genotype of a-m-1 sh/ a-m-1 sh/a-m En Sh



(1) simultaneous loss of En and Sh

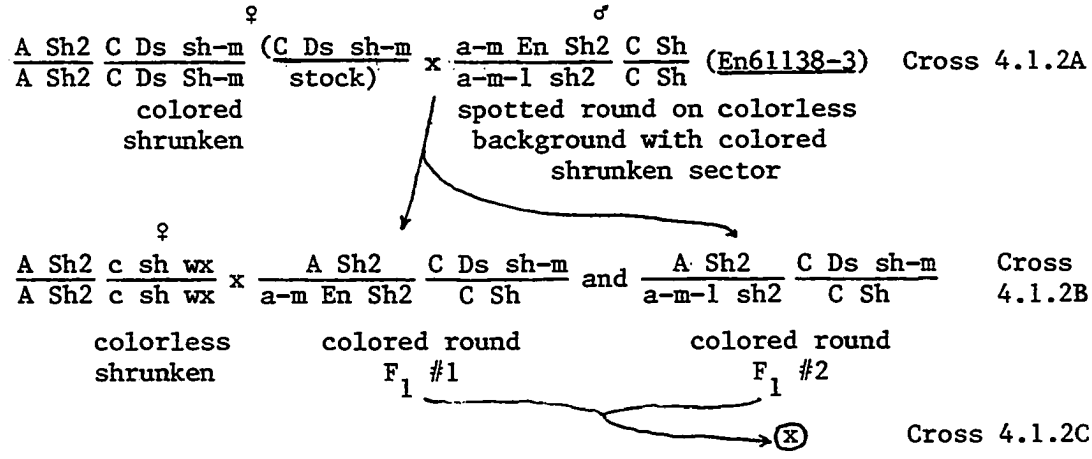


(2) simultaneous loss of a En and Sh



4.1.2. Proof that the loss event is due to En not Ac

Because the Ac element has been associated with chromosome breaks and the induction of chromosome loss, the remote possibility arose that this multiple gene loss event of the a En Sh segment on chromosome 3 might have arisen from the insertion of an Ac element within the a locus. To test for the presence of Ac as the inducer of this loss event, the following crosses were executed in order to provide a Ds that will expose the presence of Ac:



En61138-3 (a-m En Sh2/a-m-1 sh2) was crossed to a C Ds sh-m stock (Cross 4.1.2A) yielding colored F_1. Half of the F_1 are expected to have both C Ds sh-m and a-m En Sh2 (En61138-3). If Ac is present, crosses of the F_1 C Ds sh-m/C Sh, A Sh2/a-m En Sh2 onto the c sh wx/c sh wx tester (Cross 4.1.2B) would express Ac because of the expected loss of the C Ds segment. At the same time, the F_1 plants derived from Cross 4.1.2A are selfed (Cross 4.1.2C) to verify the presence of En61138-3. Therefore, these two series of crosses (Crosses 4.1.2B and 4.1.2C), tests onto c sh wx tester and selfs, are designed to provide proof for the presence or absence

of Ac and the concomitant presence or absence of a-m En Sh2 loss events. The results are shown in Table 4.2.

From these crosses of the F_1's (#1 and #2) on the c sh wx tester plants (Cross 4.1.2B), two major distinguishable genotypes expressing sh1 phenotype are expected: A Sh2/a-m En Sh2 C Ds sh-m/c sh (colored shrunken) from cross of F_1 #1 and A Sh2/a-m-1 sh2 C Ds sh-m/c sh (colored shrunken) from cross of F_1 #2. If Ac is present in cross of F_1 #1 in En61138-3, one-half of the colored shrunken kernels will be expected to show colorless sectors and sh1 to Sh1 sectors (in C Ds sh-m/c sh, breaks at Ds, causes C Ds loss and sh → Sh events). However, in no case are colorless sectors found on colored shrunken kernels (Table 4.2A).

The data from the self of these F_1 plants are shown in Table 4.2B. In six out of the twelve selfed progeny, the following ratio with respect to the total number of kernels for each selfed progeny are:

Proportion of the total number of kernels	Genotype	Phenotype
9/16	4 A Sh2/a-m En Sh2 C Ds sh-m/C Sh 2 A Sh2/A Sh2 C Ds sh-m/C Sh 2 A Sh2/a-m En Sh2 C Sh/C Sh 1 A Sh2/A Sh2 C Sh/C Sh	Colored round
3/16	A Sh2/a-m En Sh2 C Ds sh-m/C Ds sh-m	Colored shrunken (sh1)
3/16	a-m En Sh2/a-m En Sh2 C Ds sh-m/C Sh	Spotted round with colorless background
1/16	a-m En Sh2/a-m En Sh2 C Ds sh-m/C Ds sh-m	Spotted shrunken (sh1) with colorless background

Table 4.2. Phenotypic frequencies of the F_1 [a-m En Sh2/a-m-1 sh2 C Sh/C Sh (En61138-3) x A Sh2/A Sh2 C Ds sh-m/C Ds sh-m (C Ds sh-m)] selfed progeny and the crosses of the same F_1's on a c sh wx/c sh wx tester

	A Outcross onto c sh wx/c sh wx				B Selfed progeny							
		Colored			Round		Shrunken					
							Colored		Colorless spotted		χ^2-test	
Line	Progeny ear 1983	Round	Shrunken (sh1)	χ^2-test (1:1)	Colored	Colorless spotted	sh1	sh2	sh1	sh2	9:3:3:1	9:3:4
1	1224-1	175	142	ns[a]	147	65	42		12		ns	
2	1224-2	175	201	ns	228		110	108				**
3	1224-4	148	192	ns	142	61	46		21	ns		
4	1224-5	96	89	ns	310	4	87	80				**
5	1224-11	91	76	ns	106	41	37		17		ns	
6	1224-12	25	28	ns	225	77	60		21		ns	
7	1224-13	88	77	ns	191		55	80				ns
8	1224-14	151	133	ns	223	76	63		22		ns	
9	1224-15	169	155	ns	186		75	94				ns
10	1359-4	173	167	ns	198	85	75		36		ns	
11	1359-6	43	30	ns	167		50	59				ns
12	1359-9	109	93	ns	194		69	100				ns

[a] ns = Nonsignificant.

** Significant at the 1% level.

The other six of the 12 tested plants giving rise to selfed progenies are as follows:

Proportion of the total number of kernels		Genotype	Phenotype
9/16	4	A Sh2/a-m-1 sh2 C Ds sh-m/C Sh	Colored round
	2	A Sh2/A Sh2 C Ds sh-m/C Sh	
	2	A Sh2/a-m-1 sh2 C Sh/C Sh	
	1	A Sh2/A Sh2 C Sh/C Sh	
3/16	2	A Sh2/a-m-1 sh2 C Ds sh-m/C Ds sh-m	Colored shrunken (sh1)
	1	A Sh2/A Sh2 C Ds sh-m/C Ds sh-m	
4/16	2	a-m-1 sh2/a-m-1 sh2 C Ds sh-m/C Sh	Colored shrunken (sh2)
	1	a-m-1 sh2/a-m-1 sh2 C Ds sh-m/C Ds sh-m	
	1	a-m-1 sh2/a-m-1 sh2 C Sh/C Sh	

These six plants should be A Sh2/a-m-1 sh2 C Ds sh-m/C Sh without En. (It should be noted here that the sh1 phenotype is readily distinguished from the sh2 phenotype.) The presence of the expected 1/16 spotted shrunken (sh1) kernels with colorless background confirms that this En is present in the male parents. At the same time, these same plants do not show C Ds losses (colorless sectors on colored background) when used as outcrosses onto the c sh wx/c sh wx tester (Table 4.2A).

Thus, the absence of C Ds losses confirms that Ac is not a component of the En61138-3 complex. Further, from the results of the outcrosses that includes the non-En containing F_1 parents (A Sh2/a-m-1 sh2 C Ds sh-m/C Sh), no colorless sectors on the colored kernels are found (Table 4.2A, lines 2, 4, 7, 9, 11, 12). This indicates that Ac is not present elsewhere in the genome of the plants containing En61138-3.

The multiple gene loss associated with the En61138-3 which has been established early is primarily due to En and it is verified that Ac activity is not present in this genome.

When tests are made to exclude Ac activity as a factor responsible for inducing certain events, assurance must be obtained that the Ds used is responsive to Ac. Thus, it is necessary to confirm the presence of and the receptivity of Ds to Ac in the Ds parents used in Cross 4.1.2A. To assess the presence of Ds in the male parents of the outcross with c sh wx/c sh wx tester (Cross 4.1.2B), the colored shrunken (sh1) kernels used as males were selected from the progeny ear 83 1224-1 (Table 4.2A, line 1) and crossed onto an Ac line (c sh wx/c sh wx Ac/+). Two major genotypes of the colored shrunken (sh1) kernels are expected from Cross 4.1.2B: A Sh2/A Sh2 C Ds sh-m/c sh and A Sh2/a-m En Sh2 C Ds sh-m/c sh. The crosses for testing the presence of Ds in these two genotypes are shown as follows:

♀		♂	
		A Sh2/A Sh2 C Ds sh-m/c sh	Cross 4.1.2D(a)
c sh wx/c sh wx Ac/+	x	or	
		A Sh2/a-m En Sh2 C Ds sh-m/c sh	Cross 4.1.2D(b)
		↓	
		⊗	Cross 4.1.2E

If the Ds linked with C and sh-m is present in the male parents of Cross 4.1.2D, colorless sectors (C → c) and round sectors on shrunken background (sh-m → Sh) should appear on the colored shrunken kernels of these crosses. From the results, all of the four crosses showed the expected phenotype (colorless sectors on colored kernels) (Table 4.3A).

Table 4.3. Phenotypic frequencies of the crosses of the colored shrunken kernels[a] with c sh wx/c sh wx Ac/+ used as female and the selfed progeny of these colored shrunken kernels[b]

		A Outcross onto c sh wx/c sh wx Ac/+[c]				B Selfed progeny[d]		
		Shrunken (sh1)				Shrunken (sh1)		
	1983 g Progeny ear	Colored	Colored with colorless sector	Colorless	χ^2-test (1:1:2)	Colored	Colorless	χ^2-test (3:1)
1	147-1	51	58	87	ns	249	96	ns
2	147-4	25	24	40	ns	272	91	ns
3	147-5	79	71	139	ns	311	120	ns
4	147-10	52	33	103	ns	193	86	*

[a]The colored shrunken kernels were selected from the ear 83 1224-1 (Table 4.2A, line 1).

[b]Details are in the text. The outcrosses should be c sh wx/c sh wx Ac/+ x A Sh2/A Sh2 C Ds sh-m/c sh [Cross 4.1.2D(a)].

[c]The expected genotypes of this outcross are A Sh2/A Sh2 C Ds sh-m/c sh +/+ (colored shrunken): A Sh2/A Sh2 C Ds sh-m/c sh Ac/+ (colored shrunken with colorless sectors): [A Sh2/A Sh2 c sh/c sh Ac/+ and A Sh2/A Sh2 c sh/c sh +/+] (two colorless shrunken).

[d]The expected genotypes of the selfed progeny are [A Sh2/A Sh2 C Ds sh-m/C Ds sh-m + 2 A Sh2/A Sh2 C Ds sh-m/c sh] (three colored shrunken): A Sh2/A Sh2 c sh/c sh (colorless shrunken).

The expected ratio of the phenotypes and genotypes in Cross 4.1.2D are listed respectively as follows for two different genotypes of the male parents.

I. Cross 4.1.2D(a)

Genotype	Phenotype	Freq.
A Sh2/A Sh2 C Ds sh-m/c sh Ac/+	Colored shrunken (sh1) with colorless sector	1/4
A Sh2/A Sh2 C Ds sh-m/c sh +/+	Colored shrunken (sh1)	1/4
A Sh2/A Sh2 c sh/c sh Ac/+ A Sh2/A Sh2 c sh/c sh +/+	Colorless shrunken (sh1)	2/4

II. Cross 4.1.2D(b)

Genotype	Phenotype	Freq.
A Sh2/A Sh2 C Ds sh-m/c sh Ac/+ A Sh2/a-m En Sh2 C Ds sh-m/c sh Ac/+	Colored shrunken (sh1) with colorless sectors	1/4
A Sh2/A Sh2 C Ds sh-m/c sh +/+ A Sh2/a-m En Sh2 C Ds sh-m/c sh +/+	Colored shrunken (sh1)	1/4
A Sh2/A Sh2 c sh/c sh Ac/+ A Sh2/A Sh2 c sh/c sh +/+ A Sh2/a-m En Sh2 c sh/c sh Ac/+ A Sh2/a-m En Sh2 c sh/c sh +/+	Colorless shrunken (sh1)	2/4

The same expected phenotypic ratios are in Cross 4.1.2D for these two kinds of male parents. However, the selfed progeny of these male parents (Cross 4.1.2E) yield different expected ratios which are shown in the following:

III. Selfed progeny of male parents (a) in Cross 4.1.2D

	Genotype	Phenotype	Freq.
1	A Sh2/A Sh2 C Ds sh-m/C Ds sh-m	Colored shrunken (shl)	3/4
2	A Sh2/A Sh2 C Ds sh-m/c sh		
1	A Sh2/A Sh2 c sh/c sh	Colorless shrunken (shl)	1/4

IV. Selfed progeny of male parents (b) in Cross 4.1.2D

	Genotype	Phenotype	Freq.
4	A Sh2/a-m En Sh2 C Ds sh-m/c sh	Colored shrunken (shl)	9/16
2	A Sh2/a-m En Sh2 C Ds sh-m/C Ds sh-m		
2	A Sh2/A Sh2 C Ds sh-m/c sh		
1	A Sh2/A Sh2 C Ds sh-m/c Ds sh-m		
1	A Sh2/A Sh2 c sh/c sh	Colorless shrunken (shl)	4/16
2	A Sh2/a-m En Sh2 c sh/c sh		
1	a-m En Sh2/a-m En Sh2 c sh/c sh		
1	a-m En Sh2/a-m En Sh2 C Ds sh-m/C Ds sh-m	Spotted shrunken (shl) with colorless background	3/16
2	a-m En Sh2/a-m En Sh2 C Ds sh-m/c sh		

The observed values of selfed progeny are presented in Table 4.3B. They are fitted in the 3:1 ratio for colored shrunken:colorless shrunken. In comparison with the results of outcrosses (Table 4.3A), it is evident that all of the male parents involved in the outcross with c sh wx/c sh wx Ac/+ have the genotype of A Sh2/A Sh2 C Ds sh-m/c sh.

From these observations, it confirms that the Ds is definitely present in the male parents of the outcrosses with c sh wx/c sh wx (Cross 4.1.2B) in Table 4.2A.

4.1.3. Genetic evidence on the extent of loss on chromosome 3

The En61138-3 allele shows a low frequency coincident multiple gene loss of the a, En, and Sh from chromosome 3 (section 4.1.1). Further genetic evidence will be given to confirm the multiple gene loss event on chromosome 3 including the A function, En, and Sh and not only En and Sh.

4.1.3.1. Test on ax-1 allele The ax-1 allele is known to be a deficiency that includes at least the a and sh gene and, in addition, a chlorophyll controlling segment. Crosses were designed to expose the extent of multiple gene loss. The ax-1/a-m-1 sh plants were crossed by plants containing the En61138-3 allele,

ax-1/a-m-1 sh x a-m En Sh/a-m-1 sh , Cross 4.1.3A

yielding two round kernel genotypes: a-m En Sh/ax-1 and a-m En Sh/a-m-1 sh. The phenotypic frequencies of the progeny of Cross 4.1.3A are shown in Table 4.4. All the colorless shrunken sectors and the colored shrunken sectors were, respectively, on the round spotted kernels with colorless background. The frequencies of colorless shrunken sectors and the colored shrunken sectors varied among the crosses. The phenotype of the spotted round kernel with colorless shrunken sectors is shown in Figure 4.3. At the same time, the ax-1/a-m-1 sh female parents of Cross 4.1.3A were crossed onto the a et/a et tester,

a et/a et x ax-1/a-m-1 sh Cross 4.1.3B

to confirm the ax-1 containing genotype from transmission performance. All of the crosses showed that the frequencies of the round colorless kernels were less than 50%. These results are presented in Table 4.5. This confirms that the ax-1 allele is present in the female parents.

Table 4.4. Frequencies of kernel phenotypes in the progeny of ax-1/a-m-1 sh x a-m En Sh/a-m-1 sh (Cross 4.1.3A)

Crosses[a] (1981)	Round									% Loss		Shrunken
	Nonspotted		Spotted colorless									Non-spotted
	Col-ored	Color-less	No loss	Large loss cl[b]	Large loss Cl[b]	Med loss cl	Med loss Cl	Small loss cl	Small loss Cl	cl	Cl	colored
1023-7/1033-3			149				1	2	1	1.31	1.31	134
1023-10/1034-2			68						2	0	2.86	85
1023-9/1034-10			145	1				5	4	3.87	2.58	171
0939-2/1035-6			161		1		1	1	6	0.59	4.71	185
0940-6/1036-3			103					3	3	2.75	2.75	117
0940-13/1036-4			6					1		14.29	0	5
0939-8/1037-3			209			1	1	1	6	0.92	3.67	223
0940-0/1037-10			162					1	3	0.60	1.81	181
0940-1/1038-5	1	1	143	-	-	-	-	-	-	0	0	164
0940-2/1038-6			165					1		0.60	0	170
0939-4/1038-9			112					3	4	2.52	3.36	166
0940-4/1038-10			138				1	2	3	1.39	2.78	175
0939-11/1039-8			88					1	1	1.11	1.11	124
1023-3/1040-5			121					2	3	1.59	2.38	138

[a]Males 81 1033-1034 are from kernels of ear 80 2535/1857-6 (Table 4.1, line 6). Males 81 1035-1040 are from kernels of ear 80 0125/1857-9 (Table 4.1, line 9).

[b]cl = Colorless; Cl = Colored.

Figure 4.3. The kernel showing colorless shrunken sectors (→) arose from the crosses of ax-1/a-m-1 sh or ax-1/a et and a-m En Sh/ a-m-1 sh (Cross 4.1.3A and Cross 4.1.3C)

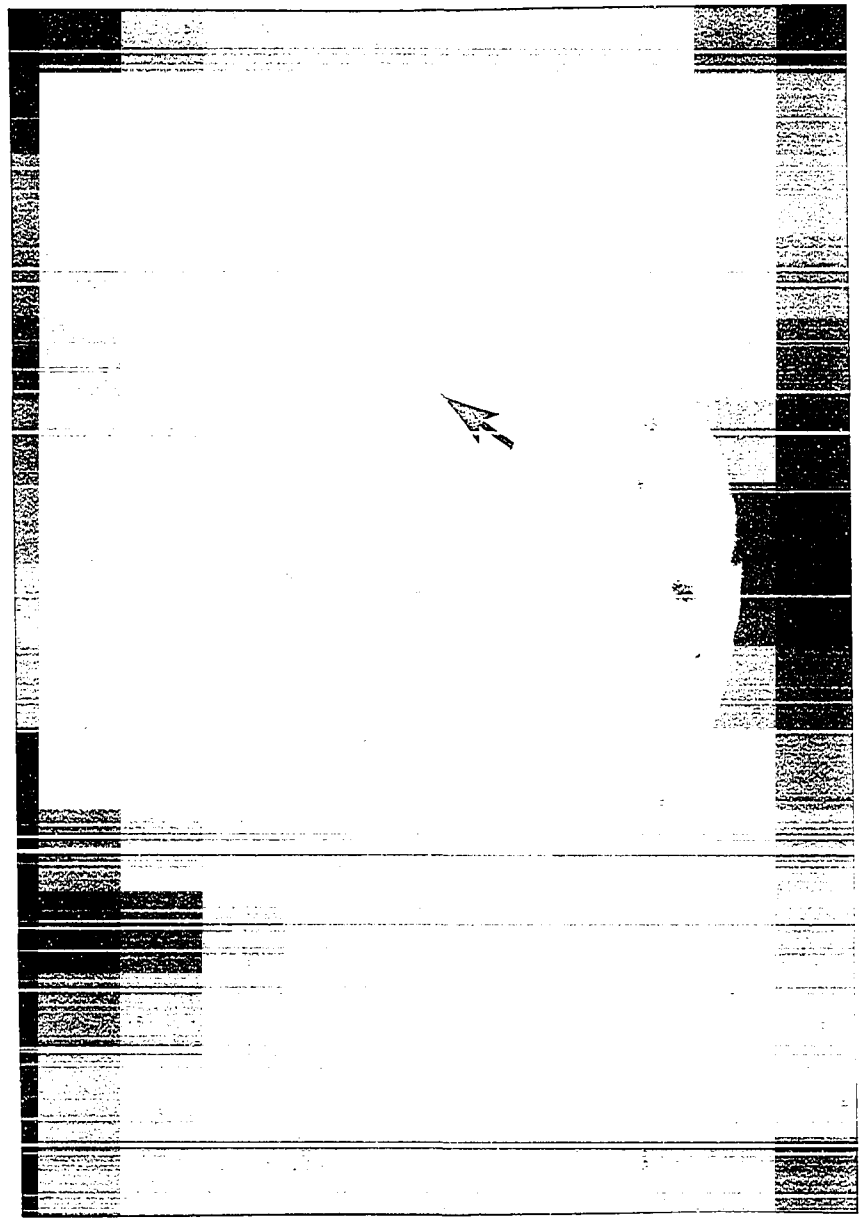


Table 4.5. Frequencies of kernel phenotypes in the progeny of a et/a et x ax-1/a-m-1 sh (Cross 4.1.3B)

Crosses (1981) ♂ number	Round		% of colorless kernels	χ^2-test (1 colored: 1 colorless)
	Colored	Colorless		
0939-2	273	125	31.41	**
-4	162	57	26.03	**
-8	106	75	41.44	*
-11	156	84	35.00	**
0940-0	180	100	35.71	**
-1	215	120	35.82	**
-2	146	98	34.82	**
-4	155	79	33.76	**
-6	87	55	38.73	**
-13	108	78	41.94	*
1023-3	87	58	40.00	*
-7	127	70	35.53	**
-9	180	100	35.71	**
-10	147	85	36.64	**

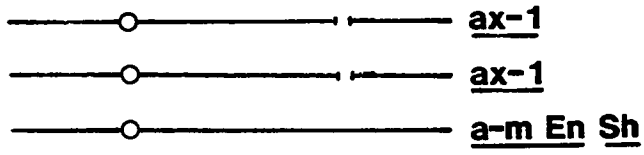
*,** Significant at the 5% and 1% levels, respectively.

The expression of the colorless shrunken sectors proves the simultaneous loss of the A function, En, and Sh in heterozygous ax-1 seeds (Figure 4.4). The genotype of the kernels with colored shrunken sectors should be a-m En Sh/a-m-1 sh which show simultaneous loss of En and Sh. These observations suggest that the extent of the loss event includes the a En Sh on chromosome 3.

Similar results were obtained from the crosses of ax-1/a et and plants containing En61138-3 allele,

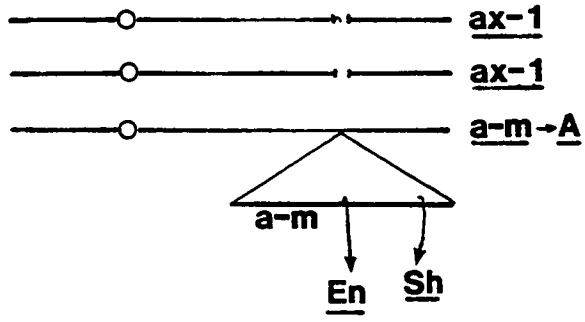
ax-1/a et x a-m En Sh/a-m-1 sh . Cross 4.1.3C

Figure 4.4. A diagram illustrating the occurrence of the loss events in the endosperm cells having the genotype of ax-1/ax-1/a-m En Sh



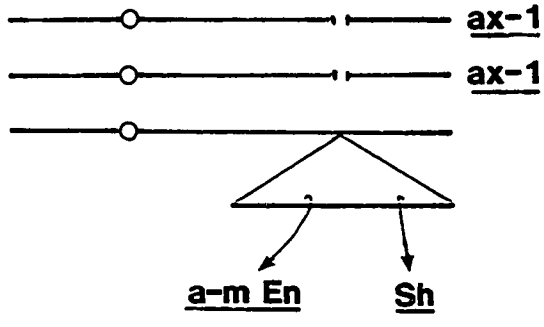
ROUND SPOTTED WITH COLORLESS BACKGROUND

(1) simultaneous loss of En and Sh



COLORED SHRUNKEN SECTORS

(2) simultaneous loss of a En and Sh



COLORLESS SHRUNKEN SECTORS

In Table 4.6, it is evident that all shrunken sectors were colorless on round spotted kernels with colorless background. Meanwhile, the ax-1/a et female parents were crossed onto a-m-1 sh/a-m-1 sh tester,

a-m-1 sh/a-m-1 sh x ax-1/a et . Cross 4.1.3D

The results are presented in Table 4.7. All the frequencies of the colored shrunken kernels were much less than the expected 50% in each cross. This confirms that the ax-1 allele is present in each of the female parents. Thus, this provides genetic evidence that it is the loss of the A function and the Sh gene that gives colorless shrunken phenotype on chromosome 3.

4.1.3.2. Test on a and sh alleles In most explanations of excision events, it is usually suggested that the element excision (En) relieves locus inhibition yielding a functional gene and that the excision is followed by restoration of gene functions, in this case, the A gene. The question remains as to what happens to the individual genes in the multiple gene loss of a, En, and Sh. This test is to identify the color of the colored shrunken sectors to be either an expression of the a-m-1 allele or that of the A gene from relief of a-m inhibition by the En insert.

It has been proved that both En and Sh is simultaneously lost in the phenotypic expression of colored shrunken sectors (section 4.1.1., 4.1.3.1). If only the En and Sh genes are lost without the coincident loss of the A function on spotted round kernels in a-m En Sh/a sh heterozygotes, the sectors are expected to be colored (A sh). To ascertain whether the A function is also lost coincident with the loss of En and Sh from chromosome 3 rather than an En excision leaving a residue of A, the

Table 4.6. Frequencies of kernel phenotypes in the progeny of ax-1/a et x a-m En Sh/a-m-1 sh (Cross 4.1.3C)

Crosses (1981)	Round						% with sector	Shrunken Colored non-spotted
	Nonspotted		Spotted					
				Colorless				
	Colored	Colorless	No loss	Large loss	Med. loss	Small loss		
A[a]								
0932-2/1032-4	79		142		1	11	7.79	68
0934-12/1033-4	108		231			8	3.35	117
0936-14/1034-9	74		178			3	1.66	77
B								
0931-4/1035-8	31		55	1		2	5.17	37
0932-6/1036-1	37		61	-	-	-	0	44
0932-10/1036-2	53		83		1	4	5.68	42
0933-5/1036-3	15		36	-	-	-	0	29
0933-1/1036-6	63	1	127			2	1.55	69
0936-1/1036-8	9	1	14			1	6.67	4
0933-7/1037-2	69	2	159	-	-	-	0	66
0932-16t/1037-5	91		169	-	-	-	0	86

[a]A: Males 81 1032-1034 are from kernels of ear 80 2535/1857-6 (Table 4.1, line 6). B: Males 81 1035-1040 are from kernels of ear 80 0125/1857-9 (Table 4.1, line 9). C: Males 81 1041 are from kernels of ear 80 0942/1858A-1t (Table 4.1, line 18). D: Males 81 1043 are from kernels of ear 80 2536/1857-7t (Table 4.1, line 7).

Table 4.6. Continued

Crosses (1981)	Round: Nonspotted Colored	Round: Nonspotted Colorless	Round: Spotted Colorless No loss	Round: Spotted Colorless Large loss	Round: Spotted Colorless Med. loss	Round: Spotted Colorless Small loss	% with sector	Shrunken Colored non-spotted
0938-3/1037-6	120		179		1	5	3.24	80
0938-1t/1037-7	76		140			1	0.71	61
0934-15/1038-11	96		154			1	0.65	69
0932-7/1039-7	77		139	-	-	-	0	67
0937-1/1039-9	18		28			3	9.68	11
0931-10/1040-1	77	3	169	-	-	-	0	71
0931-2t/1040-2	98		201	-	-	-	0	78
0936-1/1040-3	60		113	-	-	-	0	42
0934-14/1040-6	63	1	117			2	1.68	57
0931-15/1040-7	109	2	147			5	3.29	87
0932-16t/1040-10	64		144	1			0.69	69
C								
0931-14/1041-5	103		173			2	1.14	101
0931-2t/1041-6t	14		30			1	3.23	10
D								
0937-10/1043-2	107		145			1	0.68	72

Table 4.7. Frequencies of kernel phenotypes in the progeny of a-m-1 sh/ a-m-1 sh ♀ x ax-1/a et ♂ (Cross 4.1.3D)

Crosses (1981) ♂ number	Round Colored nonspotted	Shrunken Colored nonspotted	% of shrunken kernels	χ^2-test (1:1)
0931-2t	232	128	35.56	**
-4	145	42	22.46	**
-10	180	66	26.83	**
-14	171	75	30.49	**
-15	209	58	21.72	**
0932-2t	235	104	30.68	**
-6	49	21	30.00	**
-7	256	82	24.26	**
-10	40	3	6.98	**
-16t	318	114	26.39	**
0933-1t	458	96	17.33	**
-5	106	43	28.86	**
-7	346	68	16.43	**
0934-12t	289	69	19.27	**
-14	226	50	18.12	**
-15t	312	95	23.34	**
0936-1t	112	16	12.50	**
-14t	75	33	30.56	**
0937-1t	103	48	31.79	**
-10t	147	21	12.50	**
0938-1t	253	81	24.25	**
-3	236	62	20.81	**

**Significant at the 1% level.

a sh/a sh tester plants were crossed by plants containing the En61138-3 allele,

a sh/a sh x a-m En Sh/a-m-1 sh . Cross 4.1.3E

The kernel phenotypes of these crosses are shown in Table 4.8. All the shrunken sectors on the round spotted kernels are colorless (Figure 4.5).

Figure 4.5. A kernel expressing colorless shrunken sectors (→) arose from a sh/a sh x a-m En Sh/a-m-1 sh (Cross 4.1.3E)

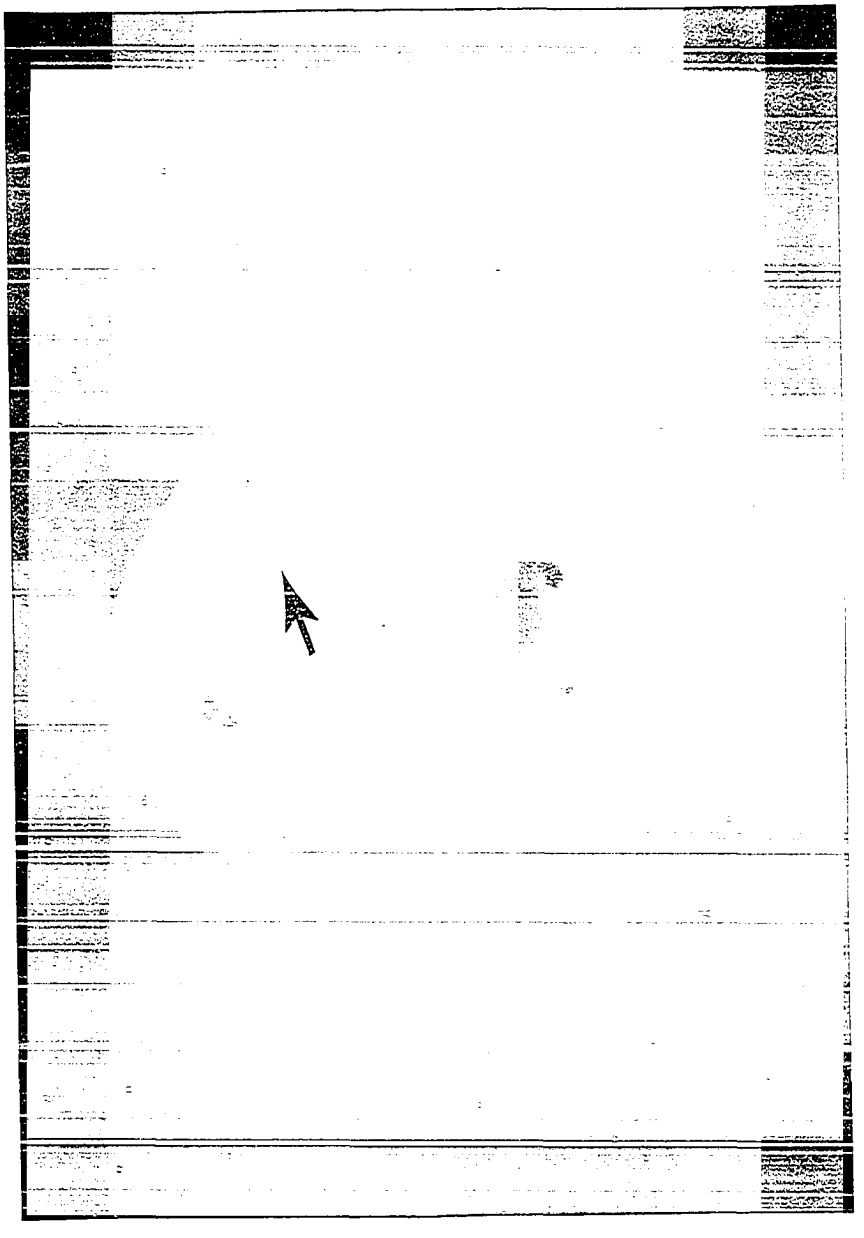


Table 4.8. Frequencies of kernel phenotypes in the progeny of a sh/a sh x a-m En Sh/a-m-1 sh (Cross 4.1.3E)

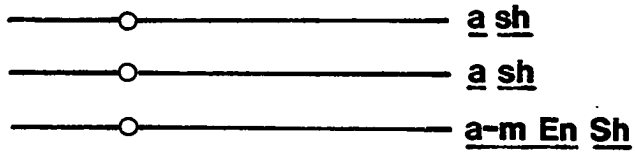
Crosses (1983) ♂ number	Round: Spotted colorless: Non-spotted colored	Round: Spotted colorless: No loss	Round: Colorless shrunken sector	Round: % sector	Shrunken: Non-spotted colored	Shrunken: Spotted colorless	χ^2-test (1 round:1 shrunken)
1334-5		174	2	1.14	154	1	ns[a]
-6		165	11	6.25	166		ns
-7		196	3	1.51	186		ns
1335-5		10	2	16.67	9		ns
-6		175	2	1.13	194		ns
-7		136	3	2.16	161		ns
-8		68	1	1.45	75		ns
-12		223	1	0.45	258		ns
1336x-1		211	2	0.93	225		ns
-3		132	1	0.75	153	1	ns
y-3		182	0	0	157		ns
1338y-6		6	2	25.00	14		ns
1339-2	1	94	1	1.05	91		ns
-5		239	1	0.42	201		ns
1341-9		40	1	2.44	36		ns
1343-1		165	2	1.20	158	1	ns
-2		89	16	15.24	105		ns
-10		152	33	17.84	197	1	ns
1344-10		143	8	5.30	171		ns

[a]ns = Nonsignificant.

This result confirms that the A function is simultaneously lost with the loss of En and Sh because there are no colored shrunken sectors (A sh) on these kernels (Figure 4.6).

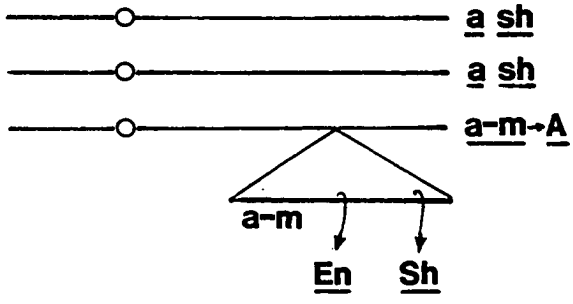
4.1.3.3. Test on the a-m-1(5720) allele Additional crosses were designed to establish the basis for the colored expression of the colored

Figure 4.6. A diagram illustrating the expected loss events occurring in the endosperm cells having the genotype, a sh/a sh/ a-m En Sh



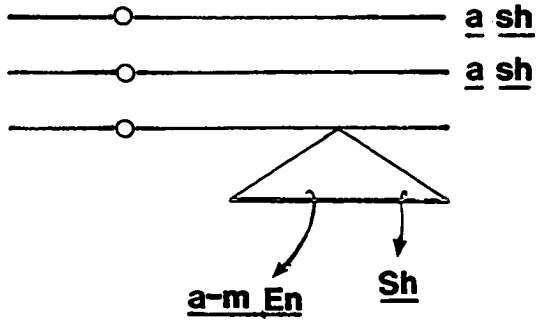
ROUND SPOTTED WITH COLORLESS BACKGROUND

(1) simultaneous loss of En and Sh



COLORED SHRUNKEN SECTORS

(2) simultaneous loss of a En and Sh



COLORLESS SHRUNKEN SECTORS

shrunken sectors as the expression of the a-m-1 allele without En rather than A gene expression arising from relief of inhibition arising from En excision. To make this test, the a-m-1(5720) allele, which is pale without En, was used. This pale is a lighter color than the a-m-1(5719) (McClintock, 1956b) and could readily be distinguished from the purple coming from the A expression. The following cross was made:

a-m-1(5720) Sh/a sh x a-m En Sh/a-m-1 sh . Cross 4.1.3F

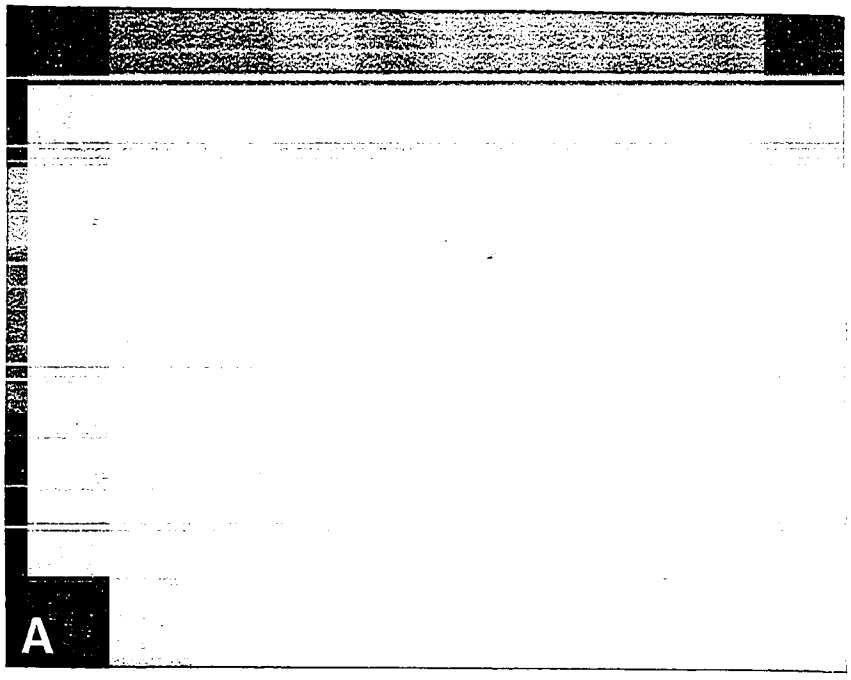
The phenotypes of the progeny of Cross 4.1.3F are shown in Table 4.9. Two kinds of sectors appeared in the round spotted kernels of this cross. There were pale colored sectors and colorless shrunken sectors expressed on the spotted round kernels with colorless background. None of the sectors was full colored (Figure 4.7A, B). From these observations, it is evident that the loss event includes the A function in addition to the En and Sh gene. Otherwise, one would expect colored sectors expressed on the round spotted kernels with a colorless background (Figure 4.8).

4.1.3.4. Use of the et locus as a distal marker to determine the extent of this multiple gene loss In order to determine the distal extent of the multiple loss event, the etched gene (et) was used as a distal marker to uncover the Et gene at 3L.91 on chromosome 3. The etched gene is pleiotropic for etched kernel and virescent seedling. From crosses of

a et/a et x a-m En Sh/a-m-1 sh , Cross 4.1.3G

3160 seedlings were tested in the greenhouse in the winter of 1981 and 1983. Only seven seedlings were found in 1983 winter that showed

Figure 4.7. Two kernels expressing the loss events arising from the crosses of a-m-1(5720) Sh/a sh x a-m En Sh/a-m-1 sh (Cross 4.1.3F)
(A) Pale sectors (→) shown on the round spotted kernel
(B) Colorless shrunken sectors (→) shown on the round spotted kernel


A

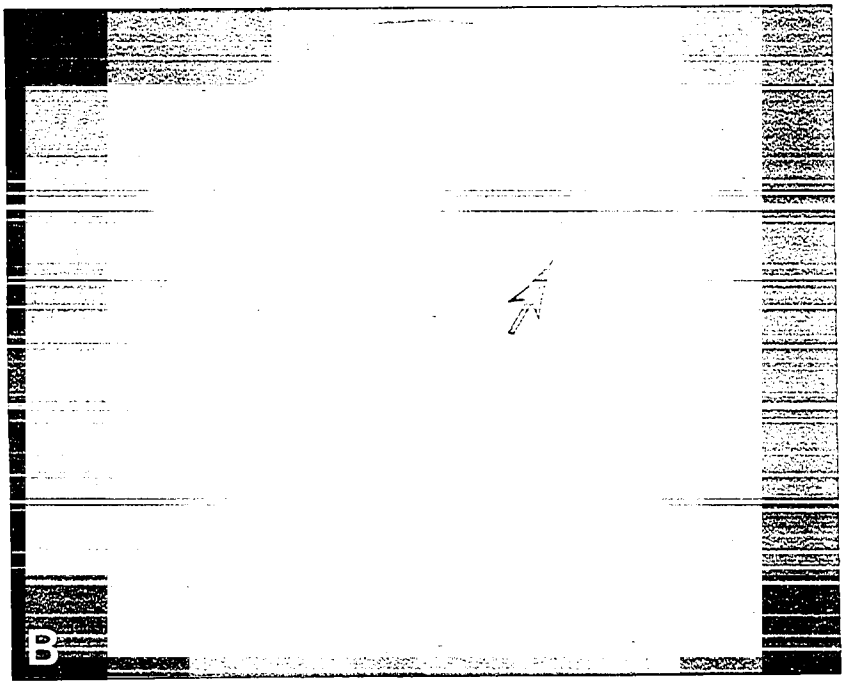
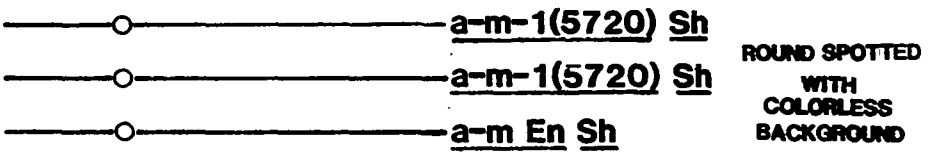
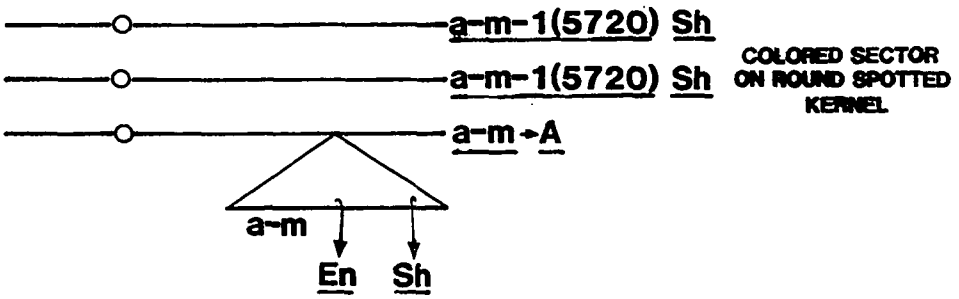

B

Figure 4.8. A diagram illustrating the loss events occurring in the endosperm cells having the genotype of a-m-1(5720) Sh/a-m-1(5720) Sh/a-m En Sh



(1) simultaneous loss of En and Sh



(2) simultaneous loss of a En and Sh

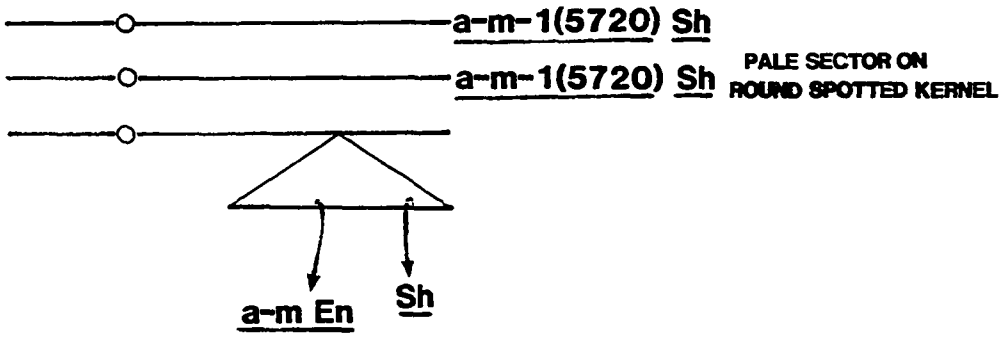


Table 4.9. Frequencies of kernel phenotypes in the progeny of a-m-1(5720) Sh/a sh x a-m En Sh/a-m-1 sh (Cross 4.1.3F)

Crosses (1983) ♂ number	Colored		Round spotted on colorless background			Colorless shrunken
	Round	Shrunken	No loss	loss (colorless shrunken sector)	Pale sector	
1241-1	100	121	195	16	32	1
1241-4	50	51	67	25	23	

virescent to white sectors on the green seedling leaves (Figure 4.9 and Table 4.10). These sectors turned to green when the seedlings grew older, a characteristic expected of the virescent plants. From these results, it is evident that the region of loss events probably extends at least to the Et locus on chromosome 3 (Figure 4.10). Whether the loss event includes the whole segment from the region of the a locus up to the end of the long arm of chromosome 3 has not been tested. The frequency of this loss from Table 4.10 is 0.22%, which is much lower than that of loss event of the En61138-3 complex on the kernels (Table 4.1).

The sequence of the developing leaves for the mutant stripes was observed from the bottom of the seedlings, and the virescent stripes only appear on the first and third leaves of the seedlings (Table 4.10).

4.1.3.5. Frequency of the loss events En61138-3 is capable of inducing the loss of a, En, and Sh from chromosome 3. This multiple gene loss can be clearly recognized by observing colored shrunken sectors on

Figure 4.9. Young seedling with virescent stripes on the green leaf of the kernel containing the genotype of a et/a-m En Sh

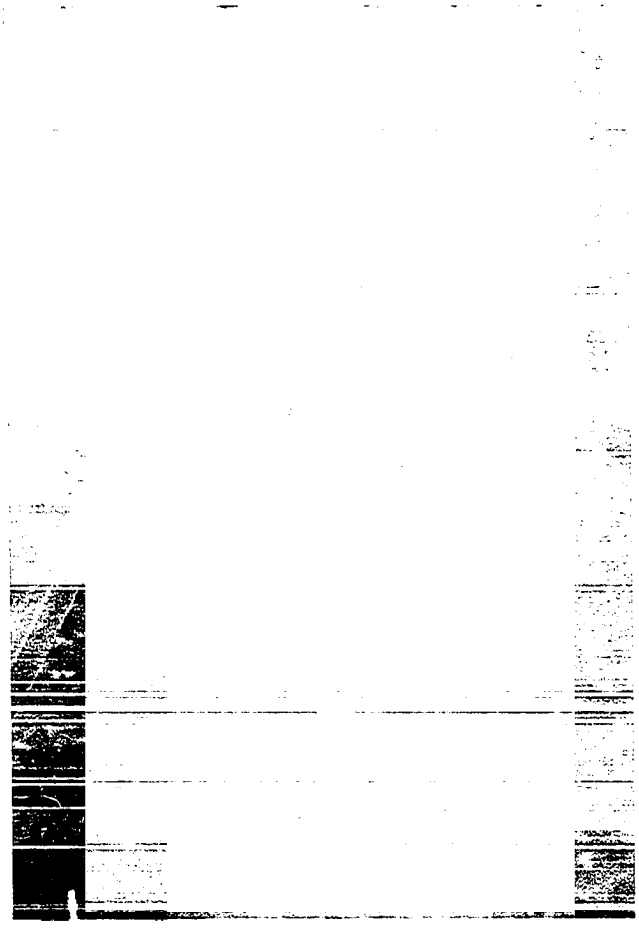


Table 4.10. Seedlings of colorless round spotted kernels from the crosses of *a et*/*a et* as female and the colorless round spotted kernels with colored shrunken sectors or without sectors as males containing the genotype of *En61138-3*/ *a-m-1 sh* (Cross 4.1.3C)

Progeny ear (male number)	No. of seedlings: Full green leaves	No. of seedlings: With virescent or white stripes	Total number seedlings	% of seedlings with virescent or white stripes
A. Seedling test in greenhouse in Winter 1981				
1. 81 1031-1	50	0	50	0
2. 81 1031-2	50	0	50	0
3. 81 10310-5	49	0	49	0
4. 81 1031-7	50	0	50	0
5. 81 1031-11	46	0	46	0
6. 81 1031-12	50	0	50	0
7. 81 1032-4	50	0	50	0
8. 81 1032-7	50	0	50	0
9. 81 1033-1	49	0	49	0
10. 81 1033-1t	50	0	50	0
11. 81 1033-5	49	0	49	0
12. 81 1033-7	49	0	49	0
13. 81 1033-8	50	0	50	0
14. 81 1033-10	50	0	50	0
15. 80 2109sib(*a et*/*a et*) ck	50[a]			
B. Seedling test in greenhouse in Winter 1983				
81 1032-7	25	0	25	0
81 1033-1t	41	1[b]	42	2.38
81 1033-8	15	0	15	0
81 1035-1	34	0	34	0
81 1036-6	23	0	23	0

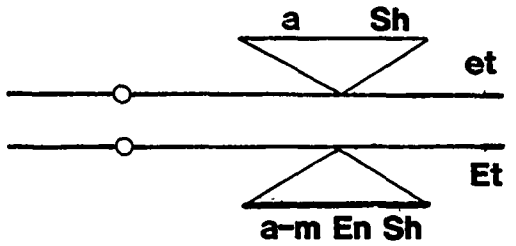
[a] All the seedlings are virescent.

[b] The occurrence of the virescent stripes/sectors on the
1. 81 1033-t -- 2nd leaf
2. 81 1038-2 -- 2nd and 3rd leaves
3. 83 1241-4 -- 3rd leaf
4. 83 1243-2 -- 3rd leaf
5. 83 1247-9 -- 1st and 2nd leaves

Table 4.10. Continued

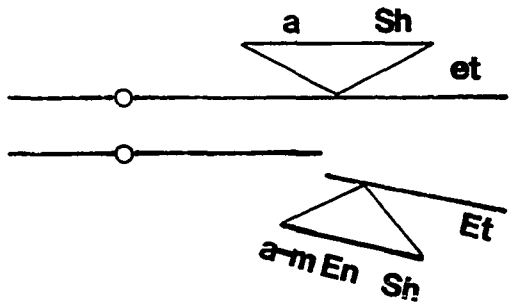
Progeny ear (male number)	No. of seedlings: Full green leaves	No. of seedlings: With virescent or white stripes	Total number seedlings	% of seedlings with virescent or white stripes
81 1036-9	60	0	60	0
81 1037-1	34	0	34	0
81 1037-2	32	0	32	0
81 1037-5	45	0	45	0
81 1037-10t	213	0	213	0
81 1037-11	50	0	50	0
81 1038-2	71	1[b]	72	1.39
81 1039-6	48	0	48	0
81 1040-2	58	0	58	0
81 1040-7	50	0	50	0
81 1040-8	99	0	99	0
81 2040-10	25	0	25	0
83 1241-4	131	2[b]	133	1.50
83 1243-2	153	1[b]	154	0.65
83 1243-3	101	0	101	0
83 1243-7	79	0	79	0
83 1245x-1	63	0	63	0
83 1245x-2	151	0	151	0
83 1247-3	103	0	103	0
83 1247-5	134	0	134	0
83 1247-9	94	2[b]	96	0
83 1249-1	121	0	121	0
83 1309-5	146	0	146	0
83 1318-3	94	0	94	0
83 1341-6	86	0	86	0
81 bulked seed	82	0	82	0
81 0957-2 ⊗ (a et/a et)	19[a]	0		
Total (A+B except ck)	3153	7	3160	0.22

Figure 4.10. Diagram showing the a En Sh Et segment lost from chromosome 3 in maize that results in the expression of a virescent phenotype on the leaves of seedlings (virescent to white stripes on the green leaves)



Et phenotype

Loss of a En Sh and Et



et phenotype

a-m-1 sh background (section 4.1.1). Therefore, it is possible to measure the frequency of multiple gene loss event in a population.

The frequency of the multiple gene loss event was measured for three different populations. The total number of aleurone cells was estimated using the Stadler estimation (1944). Parents of these three populations were derived from the kernels containing En61138-3 allele (a-m En Sh/a-m-1 sh). In the testcross population (a-m-1 sh/a-m-1 sh x a-m En Sh/a-m-1 sh), all the round spotted kernels have the a-m En Sh/a-m-1 sh/a-m-1 sh genotype for their endosperm cells; therefore, the frequency of the multiple gene loss event was measured with reference to the total number of round spotted kernels. However, the frequency of the loss event in selfed population could not be measured on the basis of the total number of round spotted kernels. The reason for this is the selfed progeny of the plants having the genotype of a-m En Sh/a-m-1 sh yield four expected endosperm genotypes. These four genotypes and their corresponding phenotypes are shown as follows:

	Genotype of endosperm	Phenotype	Frequency
1	a-m En Sh/a-m En Sh/a-m En Sh	Round spotted on colorless background	1/4
2	a-m En Sh/a-m En Sh/a-m-1 sh		1/4
3	a-m En Sh/a-m-1 sh/a-m-1 sh		1/4
4	a-m-1 sh/a-m-1 sh/a-m-1 sh	Colored shrunken	1/4

It is not possible to identify the genotypes for these round spotted kernels in this selfed population, but the multiple gene loss of a En Sh is expected to be easily seen on an a-m-1 sh background in one-third of round spotted kernels which have the genotype of a-m En Sh/a-m-1 sh/a-m-1 sh. The colored shrunken sectors might be expressed on the kernels of a-m En

Sh/a-m En Sh/a-m-1 sh genotype, but the probability of simultaneous loss of a, En, and Sh from two of three chromosomes in endosperm cells should be very rare according to the low coincident loss frequency of a-m En Sh (section 4.1.1). Therefore, it was assumed that this genotype would contribute to no loss event. Thus, in order to accomodate the difference between selfed and testcrossed population, the loss frequency estimate of the selfed population was multiplied 3X the observed frequency for the testcross population (Table 4.11, column 6). Otherwise, frequencies of selfed and testcross populations could not be compared with each other as equal components.

The results of the study of the frequency of multiple gene loss event are shown in Table 4.11 for three different populations. It was found that the rate of loss ranged from 2.3×10^{-4} to 9.8×10^{-4}. The lowest rate is in the testcross progeny of 1983. Only 2.3 cells have a chance to show the loss events in 10,000 cells for each kernel. Similar loss frequencies were found in the 1982 and 1983 selfed populations. The difference of the loss frequencies between the testcross population and the selfed population probably is due to some modifiers which will be discussed in later chapters.

4.1.3.6. Distribution of the loss events among different families of En61138-3 Six families derived from parental ears expressing different levels of loss of En61138-3 were crossed onto the a-m-1 sh/a-m-1 sh tester in 1981 (Cross 4.2.1A) in order to test for the changes in frequency of loss. The distribution of the number of the colored shrunken sectors on the single round spotted kernels having colorless background in the testcross progeny is presented in Table 4.12. Variation of loss events on

Table 4.11. Frequencies of the loss events in three different generations

Generation (1)	No. of kernels examined (2)	Average no. of cells per sector (3)	Total no. of cells in aleurone layer (4)[a]	Proportion of the cells showing loss event (5)[b]	Mean frequency of kernels with sectors per ear (%) (6)	Frequency of loss event ($X10^{-4}$) (7)[c]
1982 selfing	1087	2082.4±129	160,000	0.0130±0.0008	7.50±0.50	9.8±0.04
1983 testcross	115	1283±262	160,000	0.008±0.002	2.85±0.54	2.3±0.1
1983 selfing	169	2232.8±662	160,000	0.0140±0.004	6.10±0.50	8.5±0.2

[a]Value was estimated by Stadler (1944).

[b]Column (5) value is derived from (3)÷(4).

[c]Column (7) is the product of (5) and (6).

Table 4.12. Frequency distribution of sectors on the kernels in six families from the crosses of plants containing En61138-3 allele and a-m-1 sh/a-m-1 sh tester (Cross 4.2.1A). χ^2-values for testing the goodness of fit to a Poisson distribution are also shown in this table

No. of sectors per kernel / Family[a]	0	1	2	3	4	5	>5	Total no. of kernels	$\bar{x}$ no. of sectors per kernel	s^2	χ^2-value
1	3227	127	26	9	11	3	13	3416	.100	.2919	9483.8**
2	5448	150	7	3	0	1	2	5611	.034	.0527	261.6**
3	1579	50	7	0	1	0	0	1637	.042	.0558	27.1**
4	2384	78	9	0	0	0	0	2471	.039	.0447	31.2**
5	3527	108	13	1	0	0	0	3469	.038	.0449	79.4**
6	3180	60	2	0	0	0	0	3242	.02	.0206	3.2[ns b]
Total	19345	573	64	13	12	4	15	20026	.045	.0868	45378.9**

[a]Family 1 includes all round spotted kernels of Table 4.13. Family 2 includes all round spotted kernels of Table 4.14. Family 3 includes all round spotted kernels of Table 4.15. Family 4 includes all round spotted kernels of Table 4.16. Family 5 includes all round spotted kernels of Table. 4.17. Family 6 includes all round spotted kernels of Table 4.18.

[b]ns=Not significant.

**Significant at the 1% level.

single kernels within each family was measured by its mean square (S^2), an unbiased estimate of the variance (σ^2). These data show that the variation of the loss events is similar for families 2, 3, 4, and 5. Family 1 has the greatest variation (S^2=.2919) and Family 6 has the lowest variation (S^2=.0206). It is obvious that the one-loss per kernel events are much more frequent than the two to more than five loss events.

The Poisson distribution is the distribution of rare isolated events. It has three properties in common:

(1) the events in one time or space interval do not affect those in any other time or space interval;

(2) any given time or space interval may be subdivided successively until it contains only one event; and

(3) the conditions of the experiment remain constant over time and over space.

The occurrence of the colored shrunken sectors on round spotted kernels is a rare event (Table 4.12). Suppose that (1) these rare loss events occur independently in each kernel or in each cell; (2) the chance of the occurrence of the rare loss events remain constant and are randomly distributed in kernels or cells. Then, the Poisson distribution may be used to determine the probability of 0, 1, 2, 3, etc. loss events occurring in a given kernel or cell and to determine the randomness of the loss events in kernels (Goldstein, 1964; Schefler, 1969; Materials and Methods, section 3.7.4).

To test the goodness of fit to a Poisson distribution of loss events of En61138-3, a χ^2-test was utilized (Materials and Methods, section

3.7.4). The result is also shown in Table 4.12. The distribution of the loss events of En61138-3 does not fit the Poisson distribution in five families. The loss events in these five families have a tendency to be clustered rather than distributed randomly in kernels. It seems that some factors in addition to the principle factor, namely the En61138-3 allele itself, modify the frequency of loss events of En61138-3 in the population.

But a nonsignificant χ^2-value in Family 6 was found. This family was derived from parents with the lowest frequency of the loss event among all families. Probably the factors modifying the loss events induced by En61138-3 do not occur in this family or the number of factors is very few compared with other families.

4.2. Heritability of En61138-3

It is apparent that there is a wide range of sector size as well as a range in frequency of the loss events (ILE). Anticipating that changes in state of the ILE may occur from late events to early events or late events to no events, tests were made on the heritability of exceptional types.

4.2.1. Family basis

The percentage of colored shrunken sectors on colorless round spotted kernels (=loss event) for each ear has given rise to a different level of this loss event from a-m-1 sh/a-m-1 sh x a-m En Sh/a-m-1 sh (Cross 4.1.1B) and a-m(r) Sh/a-m-1 sh x a-m En Sh/a-m-1 sh (Cross 4.1.1C) (Table 4.1).

In order to test the heritability of these different levels of loss events, selections were made of round spotted kernels having a colorless

background with colored shrunken sectors and without colored shrunken sectors, respectively, from six parental ears showing different levels of loss events from high loss frequency to low loss frequency (28.2% to 1.7%) in 1981. Plants derived from these kernels in each family were crossed onto the a-m-1 sh/a-m-1 sh tester,

a-m-1 sh/a-m-1 sh x a-m En Sh/a-m-1 sh . Cross 4.2.1A

The results are presented in Tables 4.13 to 4.18 for each family respectively. In all these tables, it was found that the colored shrunken sectors were present in each testcross progeny among all six families. To ascertain the heritability of the En61138-3-ILE (En61138-3 induced loss event) frequency, parent-offspring correlation analysis was made and the result is shown in Figure 4.11. It indicates that there is a significant positive correlation (r=.9123*) between the frequency of the loss events of the five parental ears and that of their corresponding progenies. Owing to the unexpected effects from the extremely high loss frequency of the parental ear 80 0125/1857-9, it and its progeny were not used in the correlation analysis.

To get further confirmation on the heritability of En61138-3, similar selection and correlation procedures were done in 1982. Nine parental ears expressing a series of graded loss levels of En61138-3 were selected (16.2% to 2.4%). Plants derived from these nine ears were crossed onto the a-m-1 sh/a-m-1 sh tester,

a-m-1 sh/a-m-1 sh x a-m En Sh/a-m-1 sh . Cross 4.2.1B

The results of these testcrosses are shown in Tables 4.19 to 4.27. As with the results of the 1981 tests (Tables 4.13 to 4.18), the loss events still occurred in the testcross progenies among the nine families. The

Table 4.13. Phenotypic frequencies from crosses of colorless round spotted kernels with or without colored shrunken sectors and a-m-1 sh/a-m-1 sh tester used as female (Cross 4.2.1A)

Progeny ear (1981)	Round					Shrunken nonspotted colored	% of sector in spotted colorless kernels
	Nonspotted colored	Spotted colorless					
		No sector	Large[a] sector	Med.[a] sector	Small[a] sector		
A. Males are from kernels with sector from ear 80 2535/1857-6 (Table 4.1, line 6) (Parent A)							
Parent A		158	0	-	26	216	14.1
1. 0927/1031-1		134	0	0	3	157	2.1
2. 0912/1031-1t		51	1	0	1	64	3.8
3. 0916/1031-1t		55	0	1	1	63	3.5
4. 0929/1031-2		44	0	0	0	34	0.0
5. 0901/1031-3	2	62	0	0	3	81	4.6
6. 0903/1031-4		46	0	1	5	70	11.2
7. 0908/1031-5		29	0	0	1	52	3.3
8. 0909/1031-6		19	0	0	1	23	5.0
9. 0904/1031-7		21	0	1	5	22	22.2
10. 0928/1031-8	1	138	0	0	0	122	0.0
11. 0906/1031-10		5	0	1	1	18	28.6
12. 0930/1031-11		136	0	0	1	162	0.7
13. 0917/1031-11t		44	0	0	0	89	0.0
14. 0912/1031-12	1	188	0	2	2	128	2.1
15. 0911/1032-1		18	0	0	1	32	5.3
16. 0902/1032-3		78	0	2	5	94	8.2
17. 0919/1032-8t		103	0	1	15	130	13.5
18. 0921/1032-8t		163	3	3	18	171	12.8
19. 0911/1032-8t		81	1	7	42	143	38.2
20. 0905/1032-9		83	0	0	6	114	6.7
Total	4	1454	5	19	111	$\bar{X}$±S.E. = 8.6±2.3	

[a]Large sector is the size larger than 4 mm^2. Small sector is the size smaller than .25 mm^2. Medium sector is the size between .25 and 4 mm^2.

Table 4.13. Continued

Progeny ear (1981)	Round					Shrunken nonspotted colored	% of sector in spotted colorless kernels
	Nonspotted coloroed	Spotted colorless					
		No sector	Large sector	Med. sector	Small sector		
B. Males are from kernels without sectors from ear 2535/1857-6 (Table 4.1, line 6)							
21. 0923/1033-1t		110	0	0	2	115	2.7
22. 0910/1033-3		97	0	0	3	87	3.0
23. 0952/1033-4t		135	1	0	0	145	0.7
24. 0952/1033-4t		210	1	0	3	190	1.9
25. 0907/1033-5		79	0	1	6	103	8.1
26. 0914/1033-6t		124	0	0	0	129	0.0
27. 0930/1033-6t		168	2	1	3	181	3.5
28. 0947/1033-10	1	176	2	2	1	152	2.8
29. 0919/1033-11		30	0	0	0	31	0.0
30. 0910/1033-11t		68	0	1	3	64	5.6
31. 0902/1034-1		131	0	0	1	91	0.7
32. 0902/1034-3		188	0	1	8	207	4.6
33. 0946/1034-9		116	0	2	2	123	3.3
34. 0906/1034-10		35	0	0	5	50	12.5
35. 0924/1034-10t		62	1	0	0	62	1.6
Total	1	1729	7		37	$\bar{X}$±S.E. = 3.4±0.9	

Table 4.14. Phenotypic frequencies from crosses of colorless round spotted kernels with or without colored shrunken sectors and a-m-1 sh/a-m-1 sh tester used as female (Cross 4.2.1A)

Progeny ear (1981)	Round					Shrunken nonspotted colored	% of sector in spotted colorless kernels
	Nonspotted colored	Spotted colorless					
		No sector	Large sector	Med. sector	Small sector		
A. Males are from kernels with sector from ear 0125/1857-9 (Table 4.1, line 8) (Parent B)							
Parent B		125	5	-	45	190	28.2
1. 0904/1035-4		39	0	0	2	35	4.9
2. 0905/1035-5		10	1	0	1	6	16.7
3. 0910/1035-9		4	0	0	0	2	0
4. 0911/1035-11		155	0	0	1	153	0.6
5. 0952/1036-4t	2	118	1	0	1	126	1.7
6. 0903/1036-6		97	0	1	0	98	1.0
7. 0953/1036-6t		188	0	0	0	178	0
8. 0925/1037-1		45	0	1	2	33	6.3
9. 0915/1037-2		125	0	0	3	148	2.3
10. 0922/1037-2		132	0	0	0	183	0
11. 0930/1037-3	2	182	0	3	3	151	3.2
12. 0918/1037-5		135	0	0	3	134	2.2
13. 0918/1037-5		76	0	0	0	59	0
14. 0916/1037-6		88	1	0	2	84	3.3
15. 0930/1037-6		123	0	0	1	133	0.8
16. 0915/1037-7		161	0	4	8	154	6.9
17. 0929/1037-7		181	0	0	15	191	7.7
18. 0929/1037-8		57	0	0	0	84	0
19. 0927/1037-9		141	0	0	0	139	0
20. 0913/1037-9	2	126	0	0	0	121	0
21. 0929/1037-10		145	0	0	1	138	0.7
22. 0922/1037-10t	2	217	3	1	18	238	9.2
23. 0911/1037-11		36	0	0	0	46	0
Total	8	2647	6	10	61	$\bar{x}$±S.E. = 3.3±0.9	

Table 4.14. Continued

Progeny ear (1981)	Round					Shrunken nonspotted colored	% of sector in spotted colorless kernels
	Nonspotted colored	Spotted colorless					
		No sector	Large sector	Med. sector	Small sector		
B. Males are from kernels without sector from ear 0125/1857-9 (Table 4.1, line 8)							
24. 0909/1038-1		66	0	0	8	90	10.8
25. 0910/1038-2		79	0	0	0	92	0
26. 0904/1038-5		42	0	0	0	42	0
27. 0929/1039-1		35	0	0	1	36	2.8
28. 0926/1039-3		105	1	0	0	123	0.9
29. 0906/1039-6		133	1	0	4	132	3.6
30. 0907/1039-7		95	0	1	3	104	4.0
31. 0921/1039-7t		95	0	0	3	112	3.1
32. 0946/1039-7t		154	0	0	1	107	0.7
33. 0906/1039-11		83	0	1	0	100	1.2
34. 0922/1039-11t		101	0	0	0	93	0
35. 0912/1040-1	1	100	0	0	5	108	4.8
36. 0913/1040-1		52	0	0	1	75	1.9
37. 0912/1040-2	2	157	1	0	3	142	2.5
38. 0918/1040-2		172	1	2	4	161	3.9
39. 0924/1040-3		124	1	0	1	106	1.6
40. 0904/1040-4	1	166	0	3	4	166	4.1
41. 0925/1040-4		149	0	1	5	140	3.9
42. 0925/1040-5		39	0	0	0	44	0
43. 0926/1040-6	1	155	1	0	2	156	1.9
44. 0918/1040-7		64	0	0	2	46	3.0
45. 0907/1040-8		95	2	0	0	96	2.1
46. 0918/1040-8t		67	0	0	0	74	0
47. 0920/1040-8t		102	0	1	1	98	1.9
48. 0923/1040-8t		54	0	0	0	35	0
49. 0907/1040-9		124	0	1	4	144	3.9

Table 4.14. Continued

Progeny ear (1981)	Round					Shrunken nonspotted colored	% of sector in spotted colorless kernels
	Nonspotted colored	Spotted colorless					
		No sector	Large sector	Med. sector	Small sector		
50. 0926/1040-10		88	0	0	1	85	1.1
51. 0925/1040-11		171	0	0	1	146	0.6
Total		2801	8	10	54	$\bar{x}$±S.E. = 2.0±0.3	

Table 4.15. Phenotypic frequencies from crosses of colorless round spotted kernels with or without colored shrunken sectors and a-m-1 sh/a-m-1 sh tester used as female (Cross 4.2.1A)

Progeny ear (1981)	Round					Shrunken nonspotted colored	% of sector
	Nonspotted colored	Spotted colorless					
		No sector	Large sector	Med. sector	Small sector		
A. Males are from kernels with sector from ear 0942/1858A-1t (Table 4.1, line 18) (Parent C)							
Parent C	69	135	2	-	13	67	10.0
1. 0903/1041-2		77	0	3	4	79	8.3
2. 0923/1041-4	1	28	0	0	0	30	0
3. 0907/1041-5		40	0	0	0	59	0
4. 0913/1041-6t		84	1	0	0	82	1.2
5. 0907/1041-8		20	0	1	1	34	9.1
6. 0910/1041-9		72	0	1	6	78	8.9
7. 0919/1041-11		72	0	3	10	107	15.3
8. 0923/1041-11		21	0	0	0	10	0
9. 0911/1041-12		13	0	0	1	25	7.1
10. 0921/1041-13t		113	1	0	2	105	2.6
Total		540	2	8	24	$\bar{x}$±S.E. = 5.3±1.7	
B. Males are from kernels without sector from ear 0942/1858A-1t (Table 4.1, line 18)							
11. 0904/1042-3	64	67	0	0			4.3
12. 0908/1042-4	2	167	0	0	183		0.6
13. 0929/1042-8		40	0	0	38		0
14. 0920/1042-8	48	41	0	0			0
15. 0908/1042-9	65	68	0	1			6.9
16. 0904/1042-10	118	99	0	0			2.0
17. 0922/1042-10t	149	147	0	0			1.3
18. 0925/1042-10t		135	0	1	143		2.2
19. 0912/1042-11	110	97	0	0			3.0
20. 0923/1042-11t	204	178	1	2			2.2
Total		1039	1	4		$\bar{x}$±S.E. = 2.3±0.7	

Table 4.16. Phenotypic frequencies from crosses of colorless round spotted kernels with or without colored shrunken sectors and a-m-1 sh/a-m-1 sh tester used as female (Cross 4.2.1A)

Progeny ear (1981)	Round					Shrunken nonspotted colored	% of sector
	Nonspotted colored	Spotted colorless					
		No sector	Large sector	Med. sector	Small sector		
A. Males are from kernels with sectors from ear 2536/1857-7t (Table 4.1, line 7) (Parent D)							
Parent D	1	161	0	-	16	161	9.0
1. 0920/1043-1		138	0	2	11	148	8.6
2. 0905/1043-2		99	0	0	2	104	2.0
3. 0909/1043-3	14	68	0	0	3	120	4.2
4. 0909/1043-4		139	0	0	1	133	0.7
5. 0922/1043-5		108	0	0	3	132	2.7
6. 0924/1043-6		122	0	0	2	138	1.6
7. 0905/1043-7		56	0	0	1	29	1.8
8. 0913/1043-8t		146	1	0	2	180	2.0
9. 0922/1043-9		180	2	0	10	177	6.3
10. 0916/1043-10		132	0	4	8	161	8.3
11. 0923/1043-11t		155	2	0	6	127	4.9
Total		1343	5	6	49	$\bar{x}$±S.E. = 3.9±0.8	
B. Males are from kernels without sectors from ear 2536/1857-7t (Table 4.1, line 7)							
12. 0920/1044-1t		122	1	1	2	139	1.6
13. 0909/1044-2		20	0	0	1	30	4.8
14. 0911/1044-3		96	0	0	0	76	0
15. 0923/1044-5t		129	0	1	1	160	1.5
16. 0926/1044-6		253	0	1	6	255	2.7
17. 0906/1044-8		111	2	0	8	109	8.3
18. 0925/1044-11		155	0	0	1	166	0.6
19. 0923/1044-11t		155	0	0	2	147	1.3
Total		1041	1	3	21	$\bar{x}$±S.E. = 2.6±0.9	

Table 4.17. Phenotypic frequencies from crosses of colorless round spotted kernels with or without colored shrunken sectors and a-m-1 sh/a-m-1 sh tester used as female (Cross 4.2.1A)

Progeny ear (1981)	Round					Shrunken nonspotted colored	% of sector
	Nonspotted colored	Spotted colorless					
		No sector	Large sector	Med. sector	Small sector		
A. Males are from kernels from bulked seed with large sector[a] (Parent E)							
1. 0919/1045-1		72	0	0	2	64	2.7
2. 0923/1045-2		168	0	0	4	195	2.3
3. 0926/1045-2		93	2	0	2	90	4.1
4. 0927/1045-3		90	0	0	2	111	2.2
5. 0907/1045-4		120	1	0	8	127	7.0
6. 0929/1045-5		80	0	0	5	106	5.9
7. 0917-1045-6		151	0	1	1	163	1.3
8. 0921/1045-6t		105	0	0	3	127	2.8
9. 0924/1045-6t		164	0	0	0	213	0
10. 0924/1045-6t		112	1	0	1	124	1.8
11. 0928/1045-6t		194	0	0	5	201	2.5
12. 0949/1045-6t		164	0	0	0	138	0
13. 0907/1045-8		77	0	1	2	66	3.8
14. 0920/1045-10		113	0	1	9	143	8.1
15. 0915/1045-11t		124	0	1	10	166	8.1
16. 0950/1045-11t		122	2	0	1	114	2.4
17. 0947/1045-11t		228	0	1	8	172	3.8
Total		2177	6	5	63	$\bar{x}$±S.E. = 3.5±0.6	

[a]The kernels are bulked from ears 802534/1857-2, 2550/1857-2t, 0125/1857-4, 0125/1857-5, 0125/1857-9t, 2550-1/1857-10t, 0719/1857-3, 0948/1857-4, and 0942/1858A-1t (Table 4.1, lines 1, 2, 4, 5, 10, 11, 13, 14, 18)

Table 4.17. Continued

Progeny ear (1981)	Round: Nonspotted colored	Round: Spotted colorless: No sector	Round: Spotted colorless: Large sector	Round: Spotted colorless: Med. sector	Round: Spotted colorless: Small sector	Shrunken nonspotted colored	% of sector
B. Males are from kernels from bulked seed without sector[a]							
18. 0946/1046-1		190	0	1	3	200	2.1
19. 0920-1046-2		119	0	0	2	113	1.7
20. 0951/1046-2t		101	0	2	2	94	3.8
21. 0908/1046-3		23	1	0	0	35	4.2
22. 0924/1046-4t		135	1	0	2	131	2.2
23. 0948/1046-4t		103	0	0	2	73	1.9
24. 0947/1046-5	81	84	0	0	1		1.2
25. 0908/1046-8	101	105	0	0	7		6.3
26. 0921/1046-9		57	0	0	11	89	16.2
27. 0908/1046-10		138	1	0	3	140	2.8
28. 0909/1046-11		127	1	1	8	170	7.3
29. 0924/1046-12t		168	0	0	1	178	0.6
Total		1350	4	4	42	$\bar{x}$±S.E. = 4.2±1.2	

Table 4.18. Phenotypic frequencies from crosses of colorless round spotted kernels with or without colored shrunken sectors and a-m-1 sh/a-m-1 sh tester used as female (Cross 4.2.1A)

Progeny ear (1981)	Round					Shrunken nonspotted colored	% of sector
	Nonspotted colored	Spotted colorless					
		No sector	Large sector	Med. sector	Small sector		
A. Males are from kernels with low frequency of sectors[a] (Parent F)							
1. 0922/1047-2		146	0	0	6	178	4.0
2. 0922/1047-3		109	0	0	1	101	0.9
3. 0904/1047-5		56	0	0	1	69	1.8
4. 0919/1047-5t		61	0	0	1	71	1.6
5. 0947/1047-6		126	1	0	0	154	0.8
6. 0926/1047-6t		183	1	1	2	198	2.1
7. 0946/1047-6t		137	0	0	4	138	2.8
8. 0903/1047-7		46	0	0	0	24	0
9. 0909/1047-7		217	0	0	5	218	2.3
10. 0927/1047-7t		61	0	0	1	63	1.6
11. 0910/1047-8		20	0	0	0	20	0
12. 0919/1047-8t		13	0	0	1	30	7.1
13. 0926/1047-8t		115	0	0	0	113	0
14. 0946/1047-8t		162	1	1	0	176	1.2
Total		1452	3	2	22	$\bar{x}$±S.E. = 1.9±0.5	
B. Males are from kernels without sectors[a]							
15. 0950/1048-1	111	148	1	0	1		1.3
16. 0915/1048-1t	70	66	0	0	0		0
17. 0913/1048-2	109	125	0	0	3		2.3

[a]The kernels are bulked from ears 0125/1857-3, 0104/1858-1m, 0948/1857-4, 2131/1857-7, 4201/1857-7t, 2132/1857-11, 0944/1858-1t (Table 4.1, lines 3, 12, 14, 15, 16, 17, 19)

Table 4.18. Continued

Progeny ear (1981)	Round					
	Nonspotted colored	Spotted colorless			Shrunken nonspotted colored	% of sector
		No sector	Large sector	Med. sector	Small sector	
18. 0922/1048-3		193	0	1	6	219	3.5
19. 0911/1048-5		15	0	0	2	4	11.8
20. 0924/1048-6		187	1	0	4	186	2.6
21. 0927/1048-6t		144	0	1	3	146	2.7
22. 0902/1048-7		138	0	1	5	135	4.2
23. 0929/1048-7		162	0	1	2	144	1.8
24. 0928/1048-9		30	0	0	0	31	0
25. 0912/1048-11t		162	0	0	0	172	0
26. 0929/1048-11t		155	0	0	3	166	1.9
27. 0947/1048-11t		203	0	0	1	228	0.5
Total		1728	1	4	30	$\bar{x}$±S.E. = 2.5±0.008	

Figure 4.11. Correlation between five families and their corresponding parents for the frequency of En61138-3-ILE from the crosses of a-m-1 sh/a-m-1 sh x a-m En Sh/a-m-1 sh (Cross 4.2.1A)

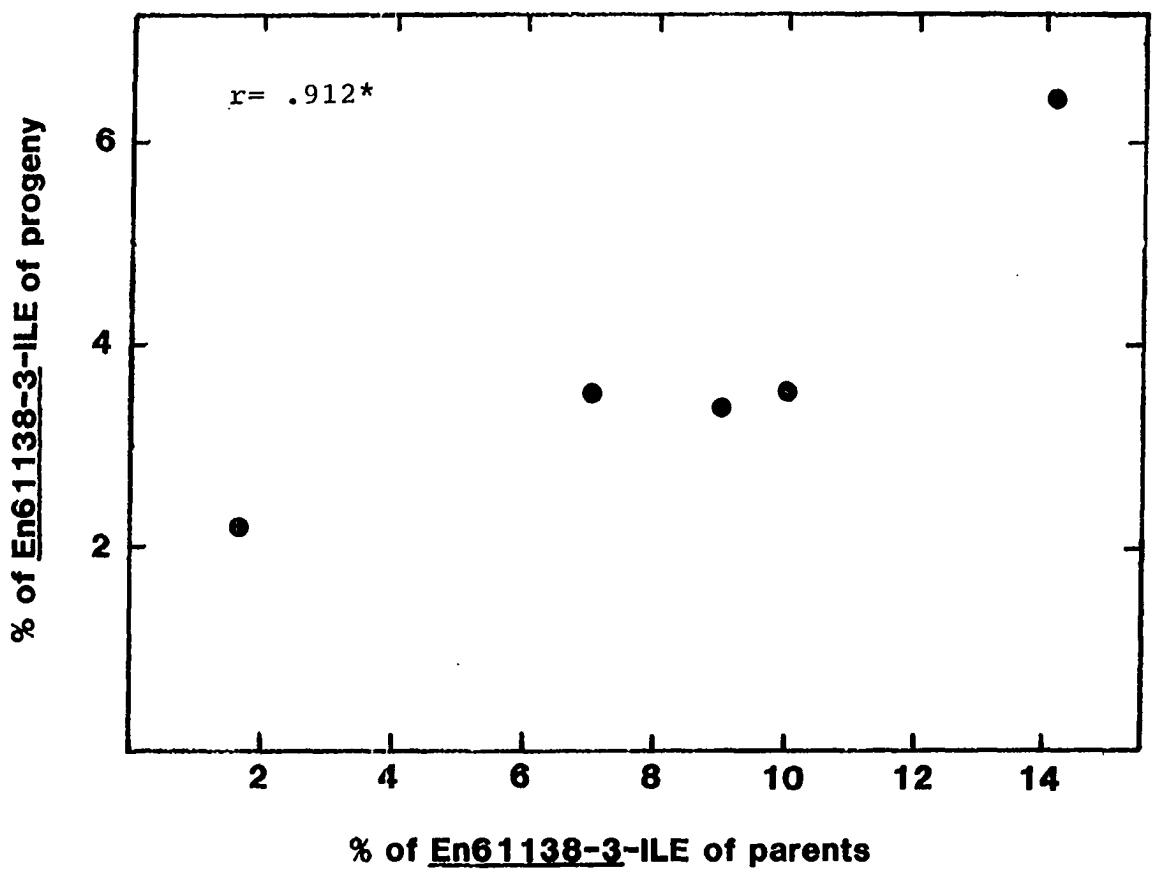

r= .912*
% of En61138-3-ILE of progeny
% of En61138-3-ILE of parents
2
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Table 4.19. Frequencies of kernel phenotypes in the progeny of a-m En Sh/a-m-1 sh crossed to a-m-1 sh/a-m-1 sh female plants (Cross 4.2.1B)

Progeny ear (1982) [male pedigree no.]	Round						Shrunken	
		Spotted colorless						
	Nonspotted colored	No sector	Large sector	Med. sector	Small sector	% of sector	Nonspotted colored	Spotted colorless
A. Males are from kernels with sector from ear 81 0919/1041-11 (Table 4.15, line 7) (Parent A2)								
1. 1141-1t		104			11	9.57	83	
2. -1t		116	2	1	8	8.66	107	
3. -2		43			5	10.42	56	
4. -2t		24			3	11.11	39	
5. -3		153		1	7	4.97	179	
6. -5t1		122		1	6	5.43	84	
7. -5t2		186			1	0.53	181	
8. -7t1		95			10	9.52	102	
9. -7t2		157	1		10	6.55	159	
10. -7t2		93		1		1.06	97	
11. -8		245	2		19	7.89	264	
12. -8t		21			1	4.55	24	
13. -9		112	1		2	2.61	95	
14. -9t		122			6	4.69	154	
B. Males are from kernels without sector from ear 81 0919/1041-11 (Table 4.15, line 7)								
15. 1142-1		43			3	6.52	57	
16. -1t1		80			2	2.44	91	
17. -1t2	1	30			1	3.23	30	
18. -2t1		81	-	-	-	0	75	
19. -2t2		160			1	0.62	162	
20. -3	1	68			6	8.11	77	
21. -5		141		1		0.70	143	
22. -5t1		130			9	6.47	151	
23. -5t2		28	1			3.45	20	

Table 4.19. Continued

Progeny ear (1982) [male pedigree no.]	Round						Shrunken	
	Nonspotted colored	Spotted colorless				% of sector	Nonspotted colored	Spotted colorless
		No sector	Large sector	Med. sector	Small sector			
24. 1142-7		210	1	2	7	4.55	258	
25. -7t		102			1	0.97	115	
26. -8		38			3	7.32	55	
27. -8t		142			3	2.07	143	

Table 4.20. Frequencies of kernel phenotypes in the progeny of a-m En Sh/a-m-1 sh crossed to a-m-1 sh/a-m-1 sh female plants (Cross 4.2.1B)

Progeny ear (1982) [male pedigree no.]	Round					Shrunken	
		Spotted colorless					
	Nonspotted colored	No sector	Large sector	Med. sector	Small sector	% of sector	Nonspotted colored	Spotted colorless
A. Males are from kernels with sector from ear 81 0921/1046-9 (Table 4.17, line 26) (Parent B2)								
1. 1143-1		175			17	8.85	165	
2. -2t		187			6	3.11	228	1
3. -3	8	96			4	4.00	66	
4. -3t		20			3	13.04	30	
5. -4		48		1	6	12.73	53	
6. -4t		71			4	5.33	86	
7. -5		137			9	6.16	144	
8. -5t		115			13	10.16	128	
9. -6		115		1	21	16.06	99	
10. -6t		159			5	3.05	202	
11. -7		81		1	10	11.96	92	1
B. Males are from kernels without sector from ear 81 0921/1046-9 (Table 4.17, line 26)								
12. 1144-1t	1	114		1	12	10.24	136	4
13. -1t	3	166		1	5	3.49	165	1
14. -2		144			16	10.00	133	1
15. -2t		66			2	2.94	86	1
16. -2t		39			6	13.33	58	
17. -3		33			1	2.94	33	
18. -3t		25			2	7.41	30	
19. -3t		138			5	3.50	136	
20. -4t		91	1		23	20.87	101	
21. -5	1	56	1		6	11.11	67	
22. -5t		94			2	2.08	132	
23. -6		62	1		2	4.62	56	

Table 4.20. Continued

Progeny ear (1982) [male pedigree no.]	Round						Shrunken	
		Spotted colorless						
	Nonspotted colored	No sector	Large sector	Med. sector	Small sector	% of sector	Nonspotted colored	Spotted colorless
24. 1144-7		30			2	6.25	31	
25. -8		122			19	13.48	122	
26. -9		17			1	5.56	26	

Table 4.21. Frequencies of kernel phenotypes in the progeny of a-m En Sh/a-m-1 sh crossed to a-m-1 sh/a-m-1 sh female plants (Cross 4.2.1B)

Progeny ear (1982) [male pedigree no.]	Round					% of sector	Shrunken	
		Spotted colorless						
	Nonspotted colored	No sector	Large sector	Med. sector	Small sector		Nonspotted colored	Spotted colorless
A. Males are from the kernels with sector from the ear 81 0920/1043-1 (Table 4.16, line 1) (Parent C2)								
1. 1145-1		64			3	4.48	65	
2. -2		40			1	2.44	38	
3. -3t2	42	25	-	-	-	0	63	
4. -4		41			3	6.82	56	
5. -5	8	182			3	1.62	180	
6. -6	4	96		1	3	4.00	118	
7. -7		74			5	6.33	55	
8. -8		14			1	6.67	12	
9. -8t		31			4	11.43	27	
10. -9		202			4	1.94	197	
11. -10		10			2	16.67	10	
12. -10		53			7	11.67	40	
13. -11	1	63			1	1.56	46	
14. -12	1	9			1	10.00	12	
B. Males are from the kernels without sector from the ear 81 0920/1043-1 (Table 4.16, line 1)								
15. 1146-1		19			2	9.52	36	
16. -2		31	-	-	-	0	32	
17. -3	1	77			2	2.53	74	
18. -4		74			2	2.63	60	
19. -4t		124			2	1.59	95	
20. -5		86	-	-	-	0	98	
21. -6		121		1	7	6.20	152	
22. -7		57	1		1	3.39	47	

Table 4.21. Continued

Progeny ear (1982) [male pedigree no.]	Round						Shrunken	
		Spotted colorless						
	Nonspotted colored	No sector	Large sector	Med. sector	Small sector	% of sector	Nonspotted colored	Spotted colorless
23. 1146-8		139	1		2	2.11	131	
24. -9		151	-	-	-	0	170	
25. -10		82	1		8	9.89	117	
26. -11	2	133			2	1.48	134	

Table 4.22. Frequencies of kernel phenotypes in the progeny of a-m En Sh/a-m-1 sh crossed to a-m-1 sh/a-m-1 sh female plants (Cross 4.2.1B)

Progeny ear (1982) [male pedigree no.]	Round						Shrunken	
		Spotted colorless						
	Nonspotted colored	No sector	Large sector	Med. sector	Small sector	% of sector	Nonspotted colored	Spotted colorless
A. Males are from the kernels with sector from ear 81 0922/1043-9 (Table 4.16, line 9) (Parent D2)								
1. 1147-1		124			4	3.13	122	
2. -1t		206			7	3.29	210	
3. -1t		58			2	3.33	64	
4. -4	1	77			1	1.28	67	
5. -6		119			4	3.25	108	
6. -8t	2	127			1	0.78	129	
7. -9		104	1		3	3.70	118	
8. -10	1	41			1	2.38	50	
B. Males are from the kernels without sector from ear 81 0922/1043-9 (Table 4.16, line 9)								
9. 1148-3t	2	165	1		6	4.07	186	1
10. -5		51	-	-	-	0	37	
11. -6		87			5	5.43	85	
12. -6t		116			4	3.33	112	
13. -7		68			2	2.86	94	
14. -7		49			2	3.92	59	
15. -7t		77			2	2.53	64	
16. -8		112	-	-	-	0	106	
17. -9	1	143	1		2	2.05	129	
18. -10		121			1	0.82	112	
19. -10t		113		1	2	2.59	109	
20. -11		62			3	4.62	54	

Table 4.23. Frequencies of kernel phenotypes in the progeny of a-m En Sh/a-m-1 sh crossed to a-m-1 sh/a-m-1 sh female plants (Cross 4.2.1B)

Progeny ear (1982) [male pedigree no.]	Round						Shrunken	
		Spotted colorless						
	Nonspotted colored	No sector	Large sector	Med. sector	Small sector	% of sector	Nonspotted colored	Spotted colorless
A. Males are from the kernels with sector from ear 81 0929/1037-7 (Table 4.14, line 17) (Parent E2)								
1. 1149-1		108			1	0.93	102	
2. -3		36			12	25.00	45	
3. -9	1	104			8	7.14	109	1
4. -10		88			1	1.12	104	
5. -11		47			2	4.08	51	
B. Males are from the kernels without sector from ear 81 0292/1037-7 (Table 4.14., line 17)								
6. 1150-1	6	29			1	3.33	46	
7. -3	5	112			3	2.61	123	
8. -3t		107	1			0.93	110	
9. -4		119	-	-	-	0	109	
10. -5		108			5	4.42	156	
11. -6	10	75			5	6.25	112	
12. -7	3	200	1		6	3.38	177	
13. -10		31	-	-	-	0	32	
14. -12	6	122		1	1	1.61	135	

Table 4.24. Frequencies of kernel phenotypes in the progeny of a-m En Sh/a-m-1 sh crossed to a-m-1 sh/a-m-1 sh female plants (Cross 4.2.1B)

Progeny ear (1982) [male pedigree no.]	Round						Shrunken	
		Spotted colorless						
	Nonspotted colored	No sector	Large sector	Med. sector	Small sector	% of sector	Nonspotted colored	Spotted colorless
A. Males are from the kernels with sector from ear 81 0902/1034-3 (Table 4.13, line 32) (Parent F2)								
1. 1151-1		109			5	4.39	76	
2. -2		139			22	13.66	181	
3. -3		87			1	1.14	71	
4. -4		200			2	0.99	180	
5. -5	1	130	2		1	2.26	136	1
6. -7		157	2		5	4.27	170	
7. -7t		184			4	2.13	132	
8. -8		22			14	38.89	38	
B. Males are from the kernels without sector from ear 81 0902/1034-3 (Table 4.13, line 32)								
9. 1152-1		142			6	4.05	129	
10. -1		93			5	5.10	123	
11. -2		168		1	1	1.18	196	
12. -3		46			2	4.17	51	
13. -4		120			1	0.83	106	2
14. -4t		47			1	2.08	37	
15. -5		185			2	1.07	132	
16. -7	2	212	2	2	3	3.20	216	2
17. -8t		164			15	8.38	191	
18. -9		58			3	4.92	55	
19. -10		72			1	1.37	92	
20. -11		215	1		14	6.52	207	

Table 4.25. Frequencies of kernel phenotypes in the progeny of a-m En Sh/a-m-1 sh crossed to a-m-1 sh/a-m-1 sh female plants (Cross 4.2.1B)

Progeny ear (1982) [male pedigree no.]	Round						Shrunken	
		Spotted colorless						
	Nonspotted colored	No sector	Large sector	Med. sector	Small sector	% of sector	Nonspotted colored	Spotted colorless
A. Males are from the kernels with sector from ear 81 0947/1045-11t (Table 4.17, line 17) (Parent G2)								
1. 1153-1		106	-	-	-	0	111	
2. -1t	1	28			4	12.50	33	1
3. -2		198			7	3.41	178	
4. -3t	1	101			4	3.81	125	1
5. -4		124			3	2.40	124	
6. -4t		81		1	2	3.57	75	
7. -5		209		1	4	2.34	195	
8. -6t	1	179	1		5	3.24	175	2
9. -7		111			5	4.31	144	
10. -8		68			4	5.56	61	
11. -9		217			3	1.36	213	
B. Males are from the kernels without sector from ear 81 0947/1045-11t (Table 4.17, line 17)								
12. 1154-1t		115			2	1.71	116	
13. -3		67		1	1	2.90	69	
14. -4		104			15	12.61	94	
15. -5		189	2		3	2.58	206	
16. -5t		137			4	2.84	152	
17. -6t		38	-	-	-	0	50	
18. -7		130			2	1.52	107	
19. -7t		128			1	0.78	137	
20. -9		106			5	4.50	129	
21. -9t		28			2	6.67	23	

Table 4.26. Frequencies of kernel phenotypes in the progeny of a-m En Sh/a-m-1 sh crossed to a-m-1 sh/a-m-1 sh female plants (Cross 4.2.1B)

Progeny ear (1982) [male pedigree no.]	Round						Shrunken	
		Spotted colorless						
	Nonspotted colored	No sector	Large sector	Med. sector	Small sector	% of sector	Nonspotted colored	Spotted colorless
A. Males are from kernels with sector from bulked seeds (low frequency of sector)[a] (Parent H2)								
1. 1155-1	6	12	-	-	-	0	11	
2. -1t	7	37			1	2.63	39	2
3. -1t2	25	80			1	1.23	122	
4. -2		127	1	1		1.55	123	
5. -2t		141	-	-	-	0	155	
6. -3		60			1	1.64	68	
7. -3t		66			3	4.35	64	
8. -4		49	-	-	-	0	60	
9. -4t		67			1	1.47	75	
10. -4t		103			1	0.96	86	
11. -4t		45	-	-	-	0	55	
12. -5	7	160	1		10	6.43	217	
13. -5t		70	1		1	2.78	57	
14. -5t2		145	1		9	6.45	165	
15. -5	1	92		1	7	8.00	124	
16. -6		89			4	4.30	78	
17. -7		94			7	6.93	107	1
18. -7t		188			1	0.53	194	
19. -7t		176			1	0.56	181	3
20. -9t		126			8	5.97	124	
21. -10		118	-	-	-	0	150	
22. -11		128		1	3	3.63	143	
23. -11t		175			4	2.23	163	

[a]The bulked seed source from 81 0927/1031-1, 0912/1031-12, 0947/1033-10 (Table 4.13, lines 1, 14, 28).

Table 4.26. Continued

Progeny ear (1982) [male pedigree no.]	Round						Shrunken	
		Spotted colorless						
	Nonspotted colored	No sector	Large sector	Med. sector	Small sector	% of sector	Nonspotted colored	Spotted colorless
B. Males are from the kernels without sector from bulked seed[a]								
24. 1156-1	35	101			1	0.98	126	
25. -4	1	126			5	3.82	155	
26. -5t	2	148			1	0.67	140	
27. -6		110	-	-	-	0	118	
28. -6t		98			3	2.97	106	
29. -9		199	1	1	2	1.97	213	
30. -9t		98	3		2	4.85	97	
31. -9t2		95			5	5.00	79	
32. -10	1	130		1	3	2.99	98	
33. -11		96			2	2.04	106	
34. -11t1		70			3	4.11	93	
35. -11t2		78	-	-	-	0	77	

Table 4.27. Frequencies of kernel phenotypes in the progeny of a-m En Sh/a-m-1 sh crossed to a-m-1 sh/a-m-1 sh female plants (Cross 4.2.1B)

Progeny ear (1982) [male pedigree no.]	Round						Shrunken	
		Spotted colorless						
	Nonspotted colored	No sector	Large sector	Med. sector	Small sector	% of sector	Nonspotted colored	Spotted colorless
A. Males are from the kernels with large sector from bulked seed[a] (Parent I2)								
1. 1157-1		35	-	-	-	0	29	
2. -1t		123			1	0.81	113	
3. -1t2	1	165			2	1.20	115	
4. -2		145			7	4.61	171	
5. -2		35			1	2.78	46	
6. -3		140	1		3	2.78	142	
7. -3t		109			1	0.91	105	
8. -5		57	1		2	5.00	53	
9. -5t		102	-	-	-	0	110	
10. -5t	2	135	-	-	-	0	115	
11. -7		139		1	5	4.14	140	
12. -8		185	1		2	1.60	53	
13. -4		214			4	1.83	191	
14. -9t	1	94			1	1.05	92	
15. -12	56	93			2	2.11	130	
B. Males are from the kernels without sector from bulked seed[a]								
16. 1158-1		74			1	1.33	53	
17. -2		141			3	2.08	138	
18. -2t		119	1	1	1	2.46	117	
19. -5		86	2		3	5.49	81	
20. -6		42			2	4.55	37	

[a]The bulked seed source from the crosses in Table 4.13, line 35; Table 4.14, lines 2, 22, 37; Table 4.15, lines 4, 20; Table 4.16, lines 8, 17; Table 4.17, line 3; Table 4.18, lines 6, 20.

Table 4.27. Continued

Progeny ear (1982) [male pedigree no.]	Round						Shrunken	
		Spotted colorless						
	Nonspotted colored	No sector	Large sector	Med. sector	Small sector	% of sector	Nonspotted colored	Spotted colorless
21. 1158-7		94	-	-	-	0		
22. -8		11	-	-	-	0	11	
23. -8t	1	32			2	5.88	31	
24. -9		27			2	6.9-	40	
25. -10		167			1	0.60	158	
26. -11	1	49			1	2.00	39	

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4.3.1. Parent-offs

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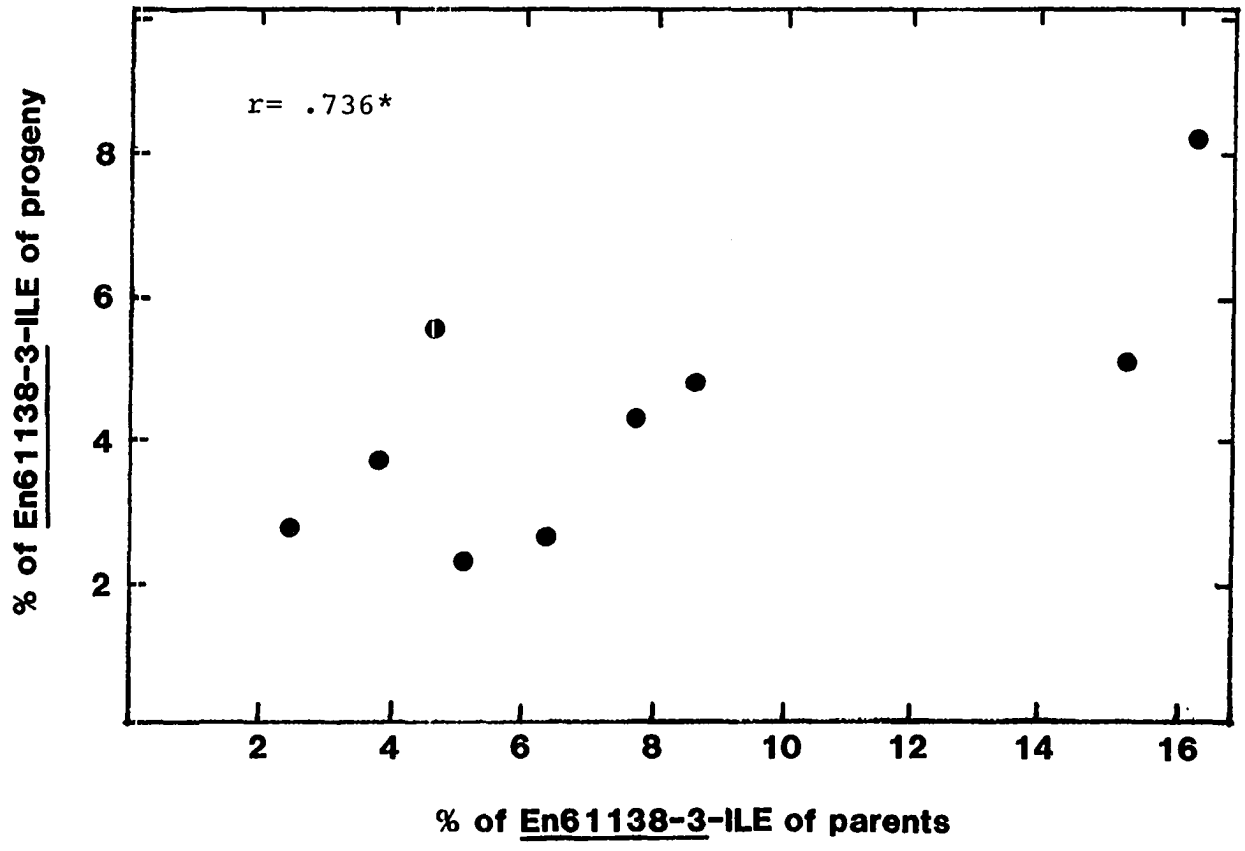
Figure 4.12. Correlation between nine families and their corresponding parents for the frequency of En61138-3-ILE from the crosses of a-m-1 sh/a-m-1 sh x a-m En Sh/a-m-1 sh (Cross 4.2.1B)


r= .736*
% of En61138-3-ILE of progeny
% of En61138-3-ILE of parents
2
4
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16

of the loss sectors in 1982 and 1983 were assigned on individual ears, respectively (Materials and Methods, section 3.3.2).

To determine the heritability of the size and the frequency of the loss sectors (states of En61138-3) on the family basis, the testcross was made on a-m-1 sh/a-m-1 sh tester plants by the plants containing En61138-3 allele (Cross 4.2.1A and Cross 4.2.1B). The same data obtained from the five families in 1981 (Tables 4.13 to 4.18, except Table 4.14) and the nine families in 1982 (Tables 4.19 to 4.27), in addition to the data collated from 1983 (Cross 4.2.1C, data not shown) were used in the correlation analysis. The results are shown in Figures 4.13 to 4.15 for 1981, 1982, and 1983, respectively.

There was a highly significant positive correlation between the states of En61138-3 of parents and that of their corresponding progeny in each year. r=.9187** in 1981, r=.9010** in 1982, and r=.8764** in 1983. These significant correlations are mainly due to the relationship between the amount of the small-sized sectors of the parental ears and that of their corresponding progeny in each year. All the "r" values for small-sized sectors between parents and their corresponding progeny in the three years showed significant or highly significant correlation (r=.8942* in 1981, r=.8272* in 1982, and r=.9037** in 1983; see Figures 4.13, 4.14, and 4.15). Only the "r" values for medium- and large-sized sectors in 1983 were highly significant between the parental ears and their corresponding progeny.

From these results, it is evident that the small-sized loss events are a highly heritable trait. The early-occurring loss events (both large sectors and medium sectors) are not heritable and this suggests

Figure 4.13. Parent-offspring correlation of states of En61138-3 for 1980-1981

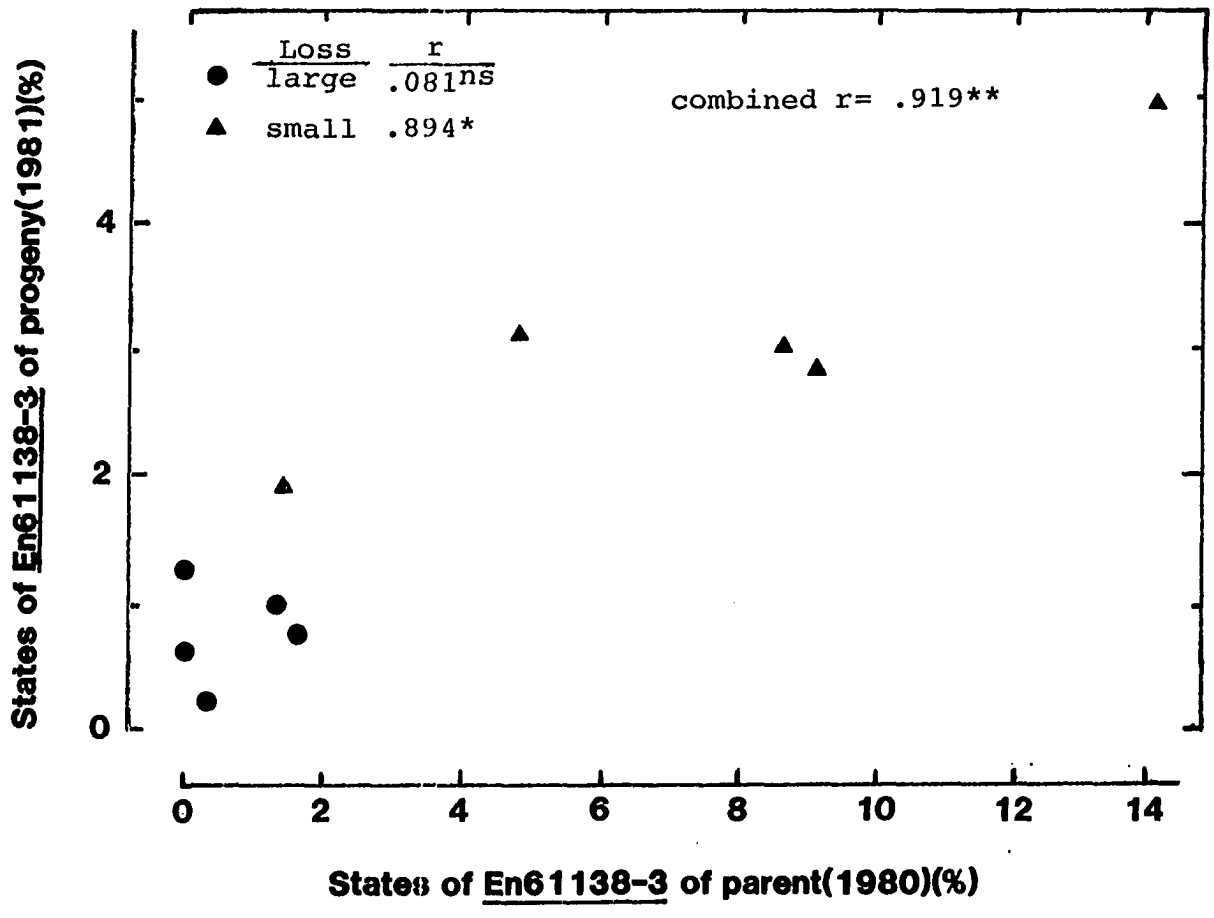

Loss
large
small
r
.081ns
.894*
combined r= .919**
States of En61138-3 of progeny(1981)(%)
States of En61138-3 of parent(1980)(%)
0
2
4
6
8
10
12
14

Figure 4.14. Parent-offspring correlation of states of En61138-3 for 1981-1982

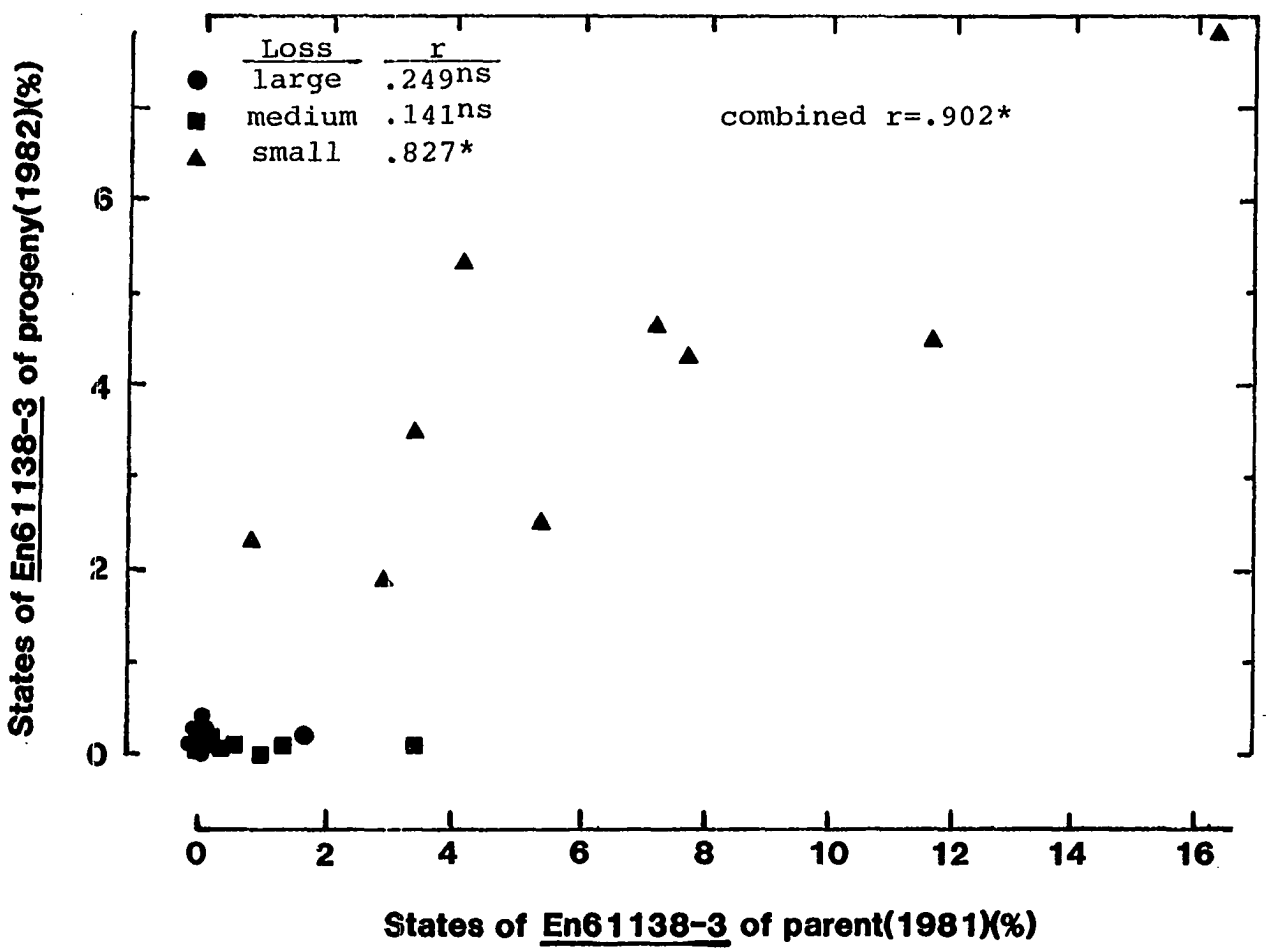
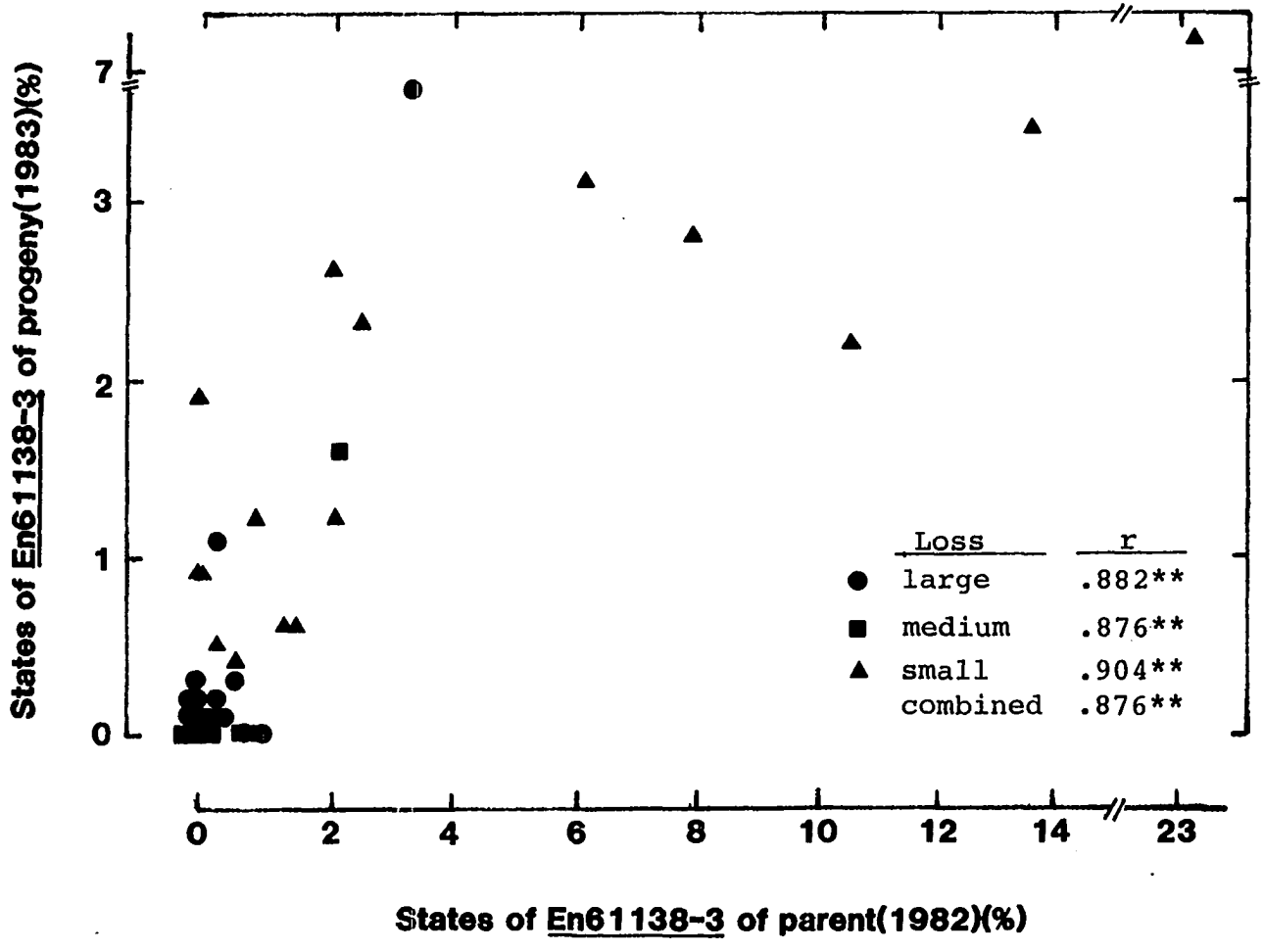

Loss
r
large .249ns
medium .141ns
small .827*
combined r=.902*
States of En61138-3 of progeny(1982)(%)
States of En61138-3 of parent(1981)(%)
0
2
4
6
8
10
12
14
16

Figure 4.15. Parent-offspring correlation of states of En61138-3 for 1982-1983


States of En61138-3 of progeny(1983)(%)
7
3
2
1
0
0
2
4
6
8
10
12
14
23
States of En61138-3 of parent(1982)(%)
Loss
r
large .882**
medium .876**
small .904**
combined .876**

that they probably occurred very rarely. Further tests on the heritability of the states of En61138-3 on single kernel basis will be given in section 4.3.2 to assess the results of this section.

4.3.1.2. Correlation between the frequency and the timing of loss events Whether progenies from ears with a high frequency of loss events are able to generate a higher frequency of early-occurring loss events is one of the questions that should be addressed.

4.3.1.2.1. Parent vs. progeny In an attempt to relate the loss frequency of the parental generation (1981 parental ears) to the frequency of the early-occurring loss events including large- and medium-sized sectors of their corresponding progeny (1982 progeny), it was found that there is no significant correlation between the parents and their progeny ($r=.5970^{ns}$) (Figure 4.16).

A similar correlation was estimated with the data of the 1982 parents and their progeny (1983). Contrary to the result of 1981-1982 correlation, it was found that there is a significant correlation ($r=.7012^{*}$) between the frequency of loss events of parental ears and the frequency of early-occurring loss events in their corresponding progeny (Figure 4.17).

Because of the homogeneity of the data of 1982 and 1983 ($\chi^2=.13^{ns}$), data of these two years could be pooled and the pooled "r" value is highly significant ($r=.6275^{**}$). These results suggest that the higher the frequency of loss events in the parental generation, the higher the frequency of early-occurring loss events are found in their progeny.

In agreement with the expectation from the results in section 4.3.1.1, the frequency of total loss events in the parental generation is highly

Figure 4.16. Correlation between the frequency of loss events in the parents and the frequency of early- or late-occurring loss events in their corresponding progeny

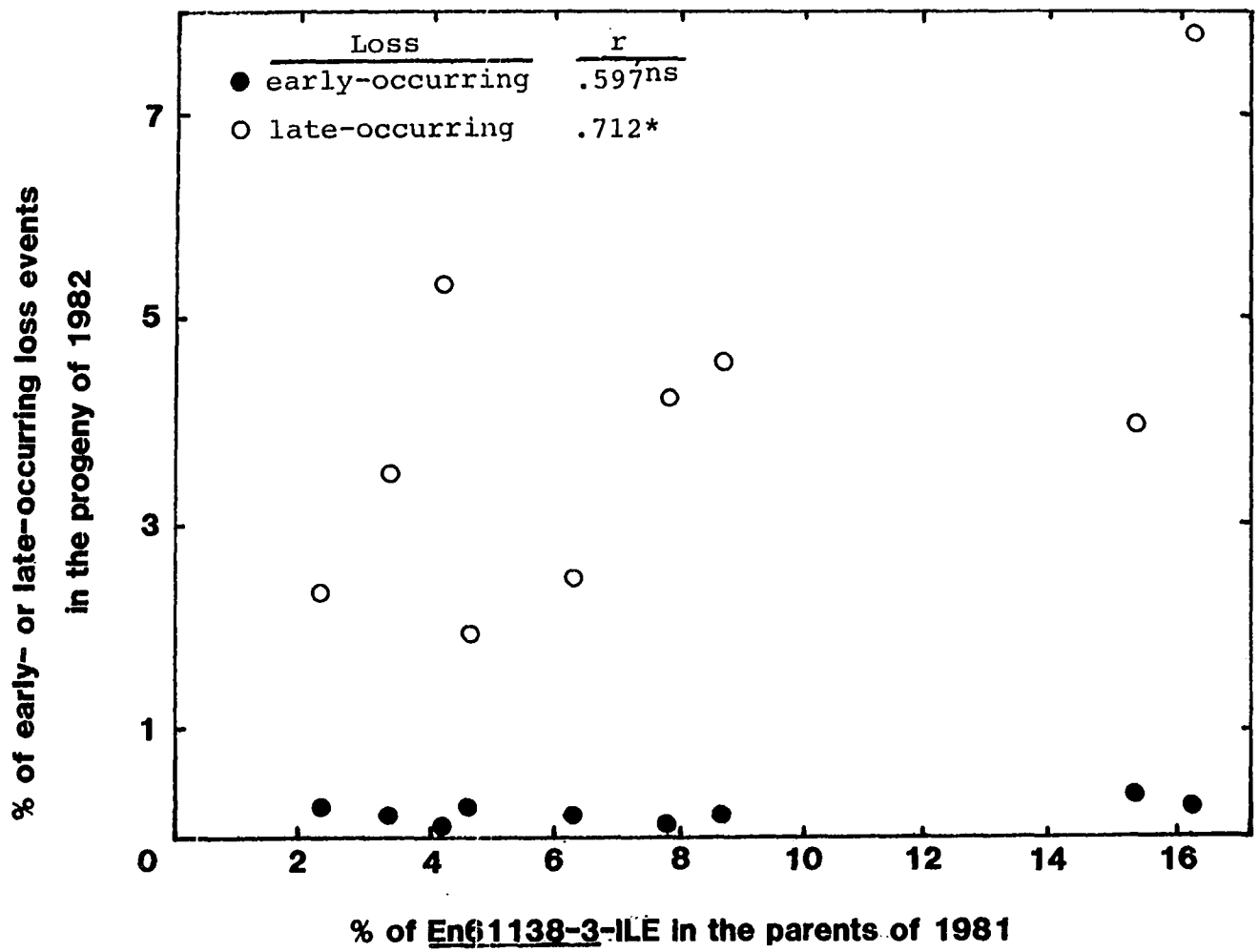
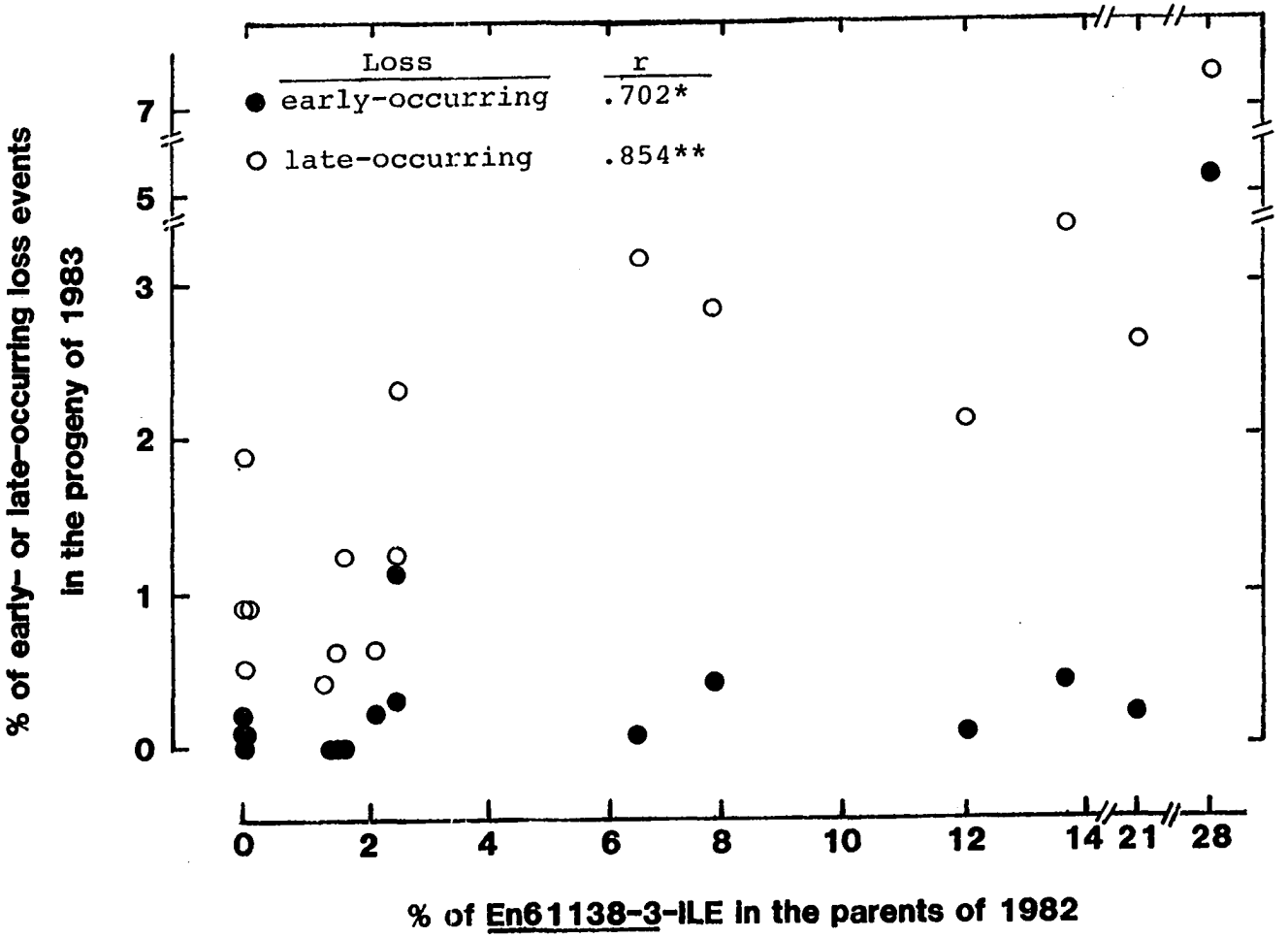

Loss
r
● early-occurring
.597ns
○ late-occurring
.712*
% of early- or late-occurring loss events in the progeny of 1982
% of En61138-3-ILE in the parents of 1981
0
1
3
5
7
2
4
6
8
10
12
14
16

Figure 4.17. Correlation between the frequency of loss events in the parents and the timing of loss events in their corresponding progeny


Loss
r
early-occurring
.702*
late-occurring
.854**
% of early- or late-occurring loss events in the progeny of 1983
% of En61138-3-ILE in the parents of 1982
0
1
2
3
5
7
0
2
4
6
8
10
12
14
21
28

correlated with the late-occurring loss events (small-sized sectors) in the progeny (r=.7116* in 1982, r=.8539* in 1983) (Figures 4.16, 4.17).

4.3.1.2.2. Progeny vs. progeny Similarly, the correlation between the frequency of total loss events and the frequency of the early-occurring loss events in the same generation was found nonsignificant in 1982, but highly significant in 1983 ($r=.2695^{ns}$ in 1982, r=.9151** in 1983) (Figures 4.18, 4.19). However, the frequency of late-occurring loss events is highly correlated with the frequency of loss events (r=.9964** in 1982, r=.9871** in 1983) in the same generation (Figures 4.18, 4.19).

Comparing the results expressed in Figures 4.16 to 4.19, it is evident that the late-occurring loss events are the major part conditioning the frequency of total loss events on an ear and it is heritable. The inconsistent correlation (example between 1982 and 1983) between the early-occurring loss events and the frequency of total loss events shows that the early-occurring loss events are rare and not readily heritable (also see Figures 4.13 to 4.15). These events are seemingly influenced by other factors apart from the En61138-3 allele itself.

4.3.1.3. Relationship between the sizes of the loss sectors and their frequencies in the same population An arbitrary assignment was made for small, medium, and large sectors with the value of 1, 2, and 3, respectively, to correlate with the sizes of loss sectors with their frequencies in the testcross population of 1983. The result indicates that the early-occurring loss events have less chance to take place compared with the late-occurring loss events. The correlation between the sizes of the loss sectors and their corresponding frequencies is highly negative (r=-.4724**) (Figure 4.20).

Figure 4.18. Correlation between the total frequency of loss events and the timing of loss events in the same generation (1982)

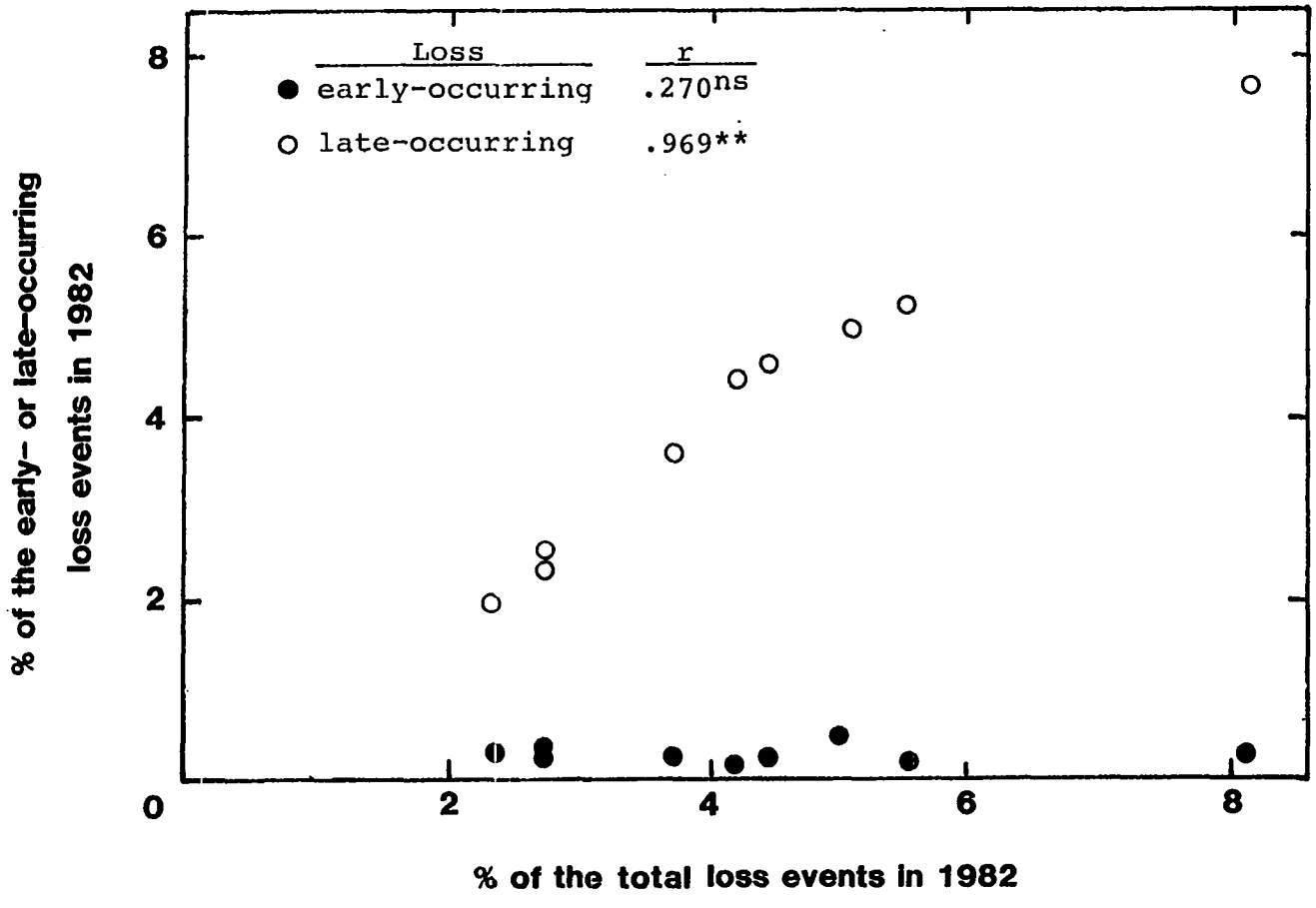

Loss
r
● early-occurring .270ns
○ late-occurring .969**
% of the early- or late-occurring loss events in 1982
% of the total loss events in 1982
0
2
4
6
8

Figure 4.19. Correlation between the percent of loss events and the percent of early- or late-occurring loss events in the same generation of 1983

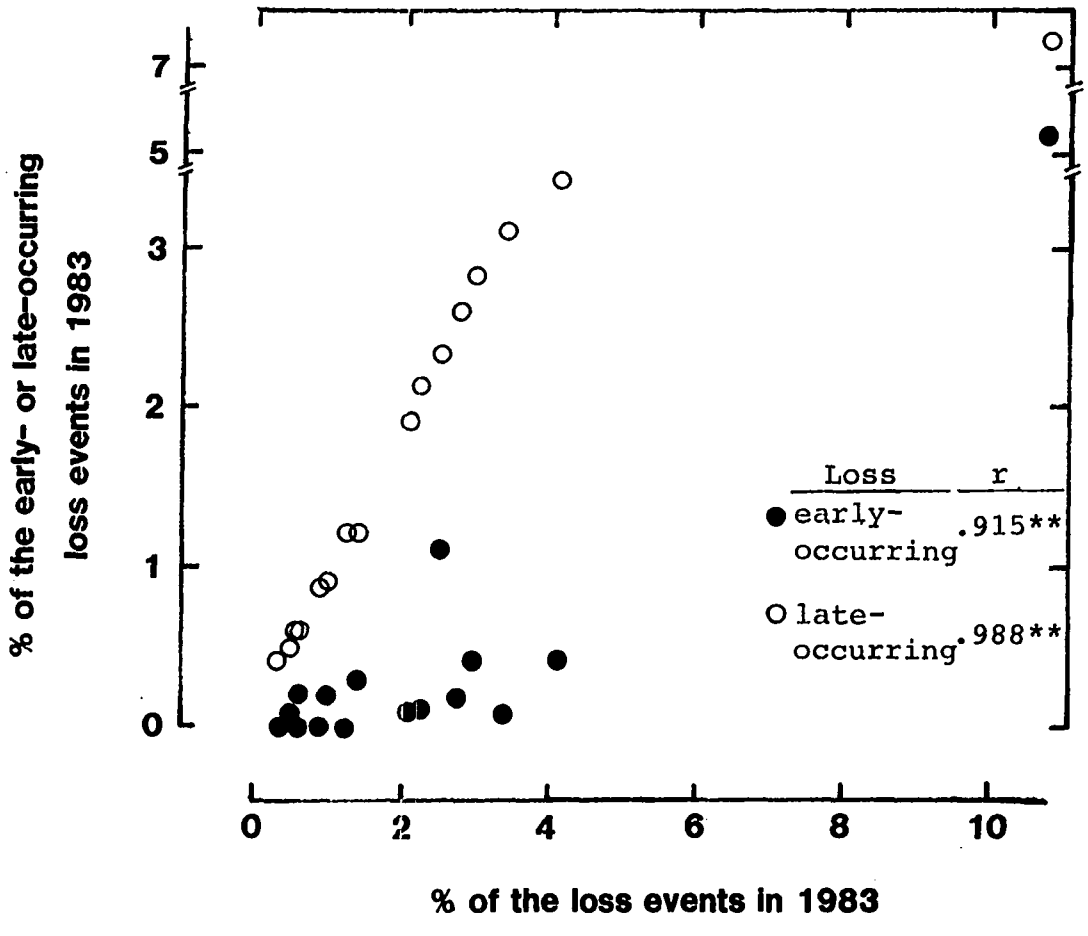
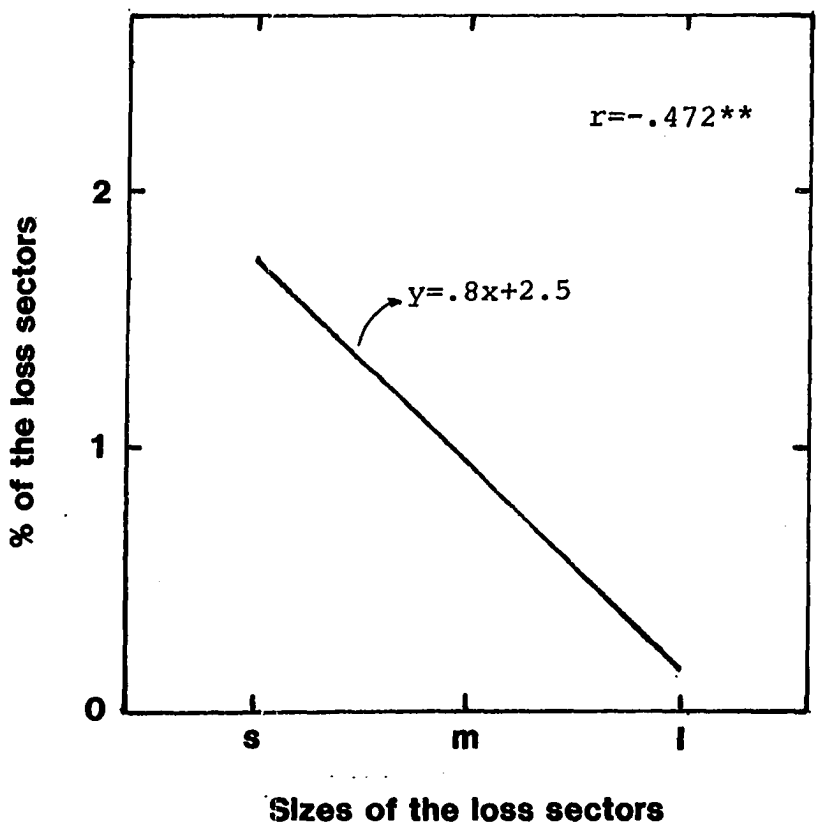

% of the early- or late-occurring loss events in 1983
7
5
3
2
1
0
0
2
4
6
8
10
% of the loss events in 1983
Loss
r
early-occurring
.915**
late-occurring
.988**

Figure 4.20. Correlation of the sizes of the loss sectors and their frequencies in the 1983 testcross progeny generation


r=-.472**
y=.8x+2.5
% of the loss sectors
2
1
0
s
m
l
Sizes of the loss sectors

Most of the loss events occur late in the development of the endosperm indicating that the timing of the occurrence of the excision of En from chromosome 3 in most of the kernels is late.

4.3.1.4. Comparison of the states of En61138-3 derived from kernels with colored shrunken sectors (+) and without colored shrunken sectors (-) Kernels having the same genotype (a-m En Sh/a-m-1 sh) were selected with (+) and without (-) colored shrunken sectors from nine parental ears in 1982 and 12 parental ears in 1983. The states of En61138-3 expressed in the testcross progeny of these families are shown in Table 4.28 and Table 4.29. The results from both tables indicate that the frequency of early-occurring loss events varied among different families between (+) and (-) kernels. But the frequency of the small sectors (late-occurring events) is consistently greater in the progeny derived from (+) kernels than that from (-) kernels.

The variability of early-occurring events in (+) and (-) kernels is probably due to the infrequent appearance of the large- or medium-sized sectors on the kernels. The differences between (+) and (-) kernels on late-occurring loss events (small sectors) are probably due to the states of En61138-3 itself or other unknown reasons.

4.3.2. Single kernel basis

Depending on the designated patterns of loss mutability (Materials and Methods, section 3.3.2), the round spotted kernels with one large sector (ℓ-1), one small sector (s-1) and kernels from the ears without any loss events (zero) were selected respectively from the Crosses 4.1.1B, 4.1.1C, 4.2.1A, and 4.2.1B (a-m-1 sh/a-m-1 sh x En61138-3/a-m-1 sh).

Table 4.28. Comparison of the states of En61138-3 derived from kernels with colored shrunken sectors (+) and without colored shrunken sectors (-) in nine families of 1982

Sizes of loss sectors \ Family[a]	1		2		3		4	
	+	-	+	-	+	-	+	-
Large	0.3±[b] 0.1	0.3± 0.3	0	0.3± 0.1	0	0.3± 0.2	0.1± 0.1	0.1± 0.1
Medium	0.2± 0.1	0.1± 0.1	0.3± 0.2	0.1± 0.1	0.1± 0.1	0.1± 0.1	0	0.1± 0.1
Small	5.8± 0.9	3.4± 0.9	8.3± 1.3	7.5± 1.3	6.0± 1.3	2.9± 1.8	2.5± 0.3	2.5± 0.5

[a]Family 1: +=82 1141 and -=82 1142 are the same as Table 4.19A, B, respectively. Family 2: +=82 1143 and -=82 1144 are the same as Table 4.20A, B, respectively. Family 3: +=82 1145 and -=82 1146 are the same as Table 4.21A, B, respectively. Family 4: +=82 1147 and -=82 1148 are the same as Table 4.22A, B, respectively. Family 5: +=82 1149 and -=82 1150 are the same as Table 4.23A, B, respectively. Family 6: +=82 1151 and -=82 1152 are the same as Table 4.24A, B, respectively. Family 7: +=82 1153 and -=82 1154 are the same as Table 4.25A, B, respectively. Family 8: +=82 1155 and -=82 1156 are the same as Table 4.26A, B, respectively. Family 9: +=82 1157 and -=82 1158 are the same as Table 4.27A, B, respectively.

[b]The values shown in this table represent the frequencies of the loss events for each size of loss sectors.

5		6		7		8		9	
+	-	+	-	+	-	+	-	+	-
0	.05± .05	0.3± .02	0.1± 0.1	.04± .04	0.1± 0.1	0.1± 0.1	0.3± 0.2	0.2± 0.1	0.2± 0.2
0	0.1± 0.1	0	0.1± 0.1	0.2± 0.1	0.1± 0.1	0.1± 0.1	0.1± 0.1	0.1± 0.1	0.1± 0.1
7.6± 4.5	2.4± 0.7	8.1± 4.6	3.3± 0.7	3.7± 1.0	3.4± 1.2	2.4± 0.3	2.1± 0.5	1.7± 0.4	2.1± 0.6

Table 4.29. Comparison of the states of En61138-3 derived from kernels with colored shrunken sectors (+) and without colored shrunken sectors (-) in 12 families of 1983

Size of loss sectors	Family[a] A		B		C		D		E		F	
	+	-	+	-	+	-	+	-	+	-	+	-
Large	2.7±[b] 0.8	1.0± 0.7	0.4± 0.4	0	0	.08± 0.8	0	3.0± 2.5	0.3± 0.1	0	0	0
Medium	1.2± 0.8	1.7± 1.0	0	0	.04± .04	0	0	0	0	.09± .09	0	0
Small	8.9± 2.2	5.5± 2.4	1.9± 0.6	0	3.1± 0.7	1.2± 0.6	3.2± 1.5	0.7± 0.7	3.2± 0.7	1.8± 0.7	0	0.8± 0.8

[a]Sources of Family A to Family L are not shown.

[b]See footnote of Table 4.28.

G		H		I		J		K		L	
+	-	+	-	+	-	+	-	+	-	+	-
0.2± 0.2	0.3± 0.3	0.4± 0.4	0	0.5± 0.4	0	0	0	0	0.2± 0.1	0	0
0.1± 0.1	0	0.1± 0.1	0	0.5± 0.4	0	0	0	0	0	0	0
4.7± 3.2	1.1± 0.5	0.6± 0.3	0.6± 0.4	5.0± 1.7	2.0± 0.8	2.1± 0.9	0.7± 0.3	3.9± 1.0	2.4± 0.4	0	0.1± 0.3

Plants derived from these kernels were again crossed onto a-m-1 sh/ a-m-1 sh tester plants (Cross 4.2.1C). The mutable patterns in the testcross progeny showed that the "s-1" state was very highly heritable. The percentage of "s-1" in the progeny of 83 1301, 83 1304, 83 1249 was 82.3%, 67.5%, and 42.9%, respectively. The average percentage is 64.3% (Tables 4.30 to 4.31). However, the heritability of the "ℓ-1" state was very low. In two testcross progeny, most of the kernels with sectors showed late-occurring loss events (s-1). The percentages of the "ℓ-1" state were only 10.5% for 81 1045 and 15.9% for 82 1157. The average percentage is 13.5% (Tables 4.33 and 4.34).

The "zero" state in the four testcross progeny of 83 1254, 83 1255, 83 1307, 83 1308 showed that its heritability is 36.4%, 14.3%, 62.5%, and 33.3%, respectively. The average percentage is 36.6% (Tables 4.35 to 4.38). These results are consistent with the results of the heritability of the states of En61138-3 on a family basis (Figures 4.13 to 4.15). The selections with late-occurring loss events are highly heritable, but those with early-occurring loss events are not heritable. The late-occurring loss events in these four "zero" state cases were largely shown to be the "s-1" type: 63.3%, 82.9%, 26.9%, and 66.7% for the progeny of 83 1254, 83 1255, 83 1307, and 83 1308, respectively, but the total frequency of En61138-3-ILE is lower in the progeny derived from "zero" than that from "s-1" or "ℓ-1" (Tables 4.30 to 4.38). From these observations, in addition to the results of Tables 4.28 and 4.29, it seems that the "zero" state is just an extreme class of the "s-1" state seeds in which no sectors happen to occur. The large-sized sectors could

Table 4.30. Heritability of the "s-1" state of En61138-3 in the testcross progeny of 83 1249 (Cross 4.2.1C)

Progeny (1983 ♂ number)	States of En61138-3[a]									Total % of loss event	% of s-1 state in the total loss events
	ℓ			s							
	1	2	3	1	2	3	4	5	6		
Parent 82 1316/1112-7 (s-1, 7.9%) (Cross 4.2.1B)											
1249-1	0[b]	0	0	0	0	0	0	0	0	0	0
-3	0	0	0	0	0	0	0	0	0	0	0
-4	-	-	-	4.0	-	-	-	-	-	4.0	100
-6	-	-	-	5.0	-	-	-	-	-	5.0	100
-7	0	0	0	0	0	0	0	0	0	0	0
-8	-	-	-	2.0	-	-	-	-	-	2.0	100
-9	-	0.8	0.8	12.4	5.0	2.5	4.2	2.5	0.8	29.0	42.8
-10	0	0	0	0	0	0	0	0	0	0	0

average % of "s-1" in the progeny = 42.9%

[a] See Figure 3.2.

[b] The values represent the frequencies (%) of each state in individual ears.

Table 4.31. Heritability of the "s-1" state of En61138-3 in the testcross progeny of 83 1301 (Cross 4.2.1C)

Progeny (1983 ♂ number)	States of En61138-3[a] ℓ 1	m 1	s 1	s 2	Total % of loss events	% of s-1 in the total loss events
Parent 82 1260/1151-2 (s-1, 13.7%) (Cross 4.2.1B)						
1301-2	0.5[b]	0.5	3.0	0	4.0	75
-4	-	-	1.0	-	1.0	100
-5	-	-	6.7	3.3	10.0	67
-7	-	-	1.0	-	1.0	100
-8	-	-	2.0	-	2.0	100
-9	3.0	3.0	6.0	6.0	18.0	33
-12	-	-	6.0	-	6.0	100
					average % of "s-1" in the progeny =	82.1%

[a]See Figure 3.2.

[b]See footnote of Table 4.30.

be an extremely infrequent loss event coexisting with late-occurring events in a limited population and these large sectors probably are a random event slipping out of the regular events. Therefore, the heritability of the early-occurring events is very low.

4.4. Factors Affecting the Behavior of En61138-3

The multiple gene loss events of En61138-3 that includes the a En Sh on chromosome 3 are due to En (section 4.1.2). There is the possibility that other factors influence this loss event. Some of the possible factors affecting the behavior of En61138-3 will be discussed in the following sections. (The En61138-3 induced loss event will be abbreviated as En61138-3-ILE in the following sections.)

Table 4.32. Heritability of the "s-l" state of En61138-3 in the testcross progeny of 83 1304 (Cross 4.2.1C)

Progeny (1983 ♂ number)	States of En61138-3[a]					Total % of loss events	% of s-l in the total loss events
	ℓ		s				
	1	5	1	2	3		
Parent 82 1308/1152-11 (s-l, 6.5%) (Cross 4.2.1B)							
1304-1	-	-	4.0	-	-	4.0	100
-2	0[b]	0	0	0	0	0	0
-6	0	0	0	0	0	0	0
-8	-	-	3.0	-	-	3.0	100
-9	-	-	7.0	-	-	7.0	100
-10	-	-	5.0	-	-	5.0	100
-11	-	-	3.3	0.7	-	4.0	82.5
-12	-	-	4.6	2.3	1.1	8.0	57.5
						average % of s-l in the progeny = 67.5%	

[a] See Figure 3.2.

[b] See footnote of Table 4.30.

4.4.1. Kinds of crosses

4.4.1.1. The frequency of the loss event in testcrosses, selfing, and sibcross progeny The frequencies of the loss events in the following types of crosses were compared in 1982.

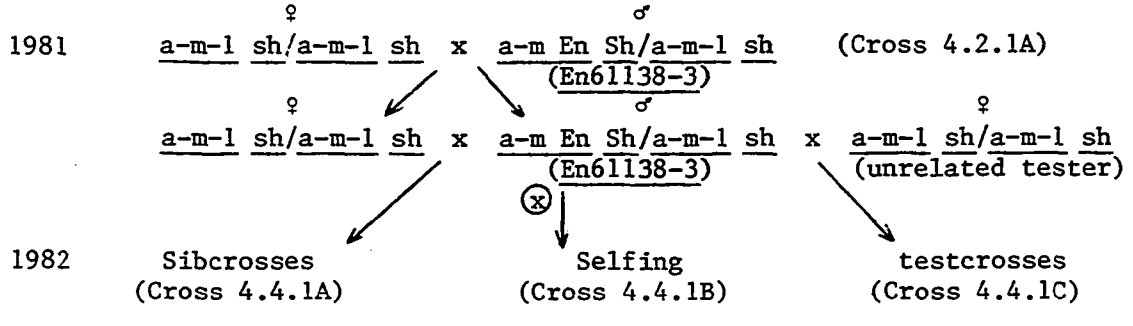


Table 4.33. Heritability of the "ℓ-1" state of En61138-3 in the testcross progeny of 81 1045 (Cross 4.2.1A)

Progeny (1981 ♂ number)	States of En61138-3[a] ℓ	m	s	Total % of the loss events	% of ℓ-1 in the total loss events
Parents 80 bulk seed (large sector $\bar{x}$=1.01%)[b] (Cross 4.1.1B and 4.1.1C)					
1045-1	-	-	2.7	2.7	0
-2	-	-	2.3	2.3	0
-2	2.0[c]	-	2.0	4.0	50
-3	-	-	2.2	2.2	0
-4	0.8	-	6.2	7.0	11.4
-5	-	-	5.9	5.9	0
-6	-	0.65	0.65	1.3	0
-6t	-	-	2.8	2.8	0
-6t	0	0	0	0	0
-6t	0.9	-	0.9	1.8	50
-6t	-	-	2.5	2.5	0
-6t	0	0	0	0	0
-8	-	1.3	2.5	3.8	0
-10	-	0.8	7.3	8.1	0
-11t	-	0.7	7.4	8.1	0
-11t	1.6	0	0.8	2.4	66.7
-11t	-	0.4	3.4	3.8	0

Average % of "ℓ-1" in the progeny = 1.05%

[a] See Figure 3.2.

[b] See footnote of Table 4.17.

[c] See footnote of Table 4.30.

Table 4.34. Heritability of the "ℓ-1" state of En61138-3 in the testcross progeny of 82 1157 (Cross 4.2.1B)

Progeny (1982 ♂ number)	States of En61138-3[a] ℓ-1	m-1	s-1	Total % of the loss events	% of ℓ-1 in the total loss events
Parent 81 bulk seed (ℓ-1, 1.64%)[b] (Cross 4.2.1A)					
1157-1t	-	-	0.8	0.8	0
-1t2	-	-	1.2	1.2	0
-2	-	-	4.6	4.6	0
-2	-	-	2.8	2.8	0
-3	0.7[c]	-	2.1	2.8	25
-3t	-	-	0.9	0.9	0
-4	-	-	1.8	1.8	0
-5	1.7	-	3.3	5.0	34
-7	-	0.7	3.4	4.1	0
-8	0.5	-	1.1	1.6	31.3
-9t	1.1	-	-	1.1	100
-12	-	-	2.1	2.1	0
				average % of "ℓ-1" in the progeny =	15.9%

[a] See Figure 3.2.

[b] See footnote of Table 4.27.

[c] See footnote of Table 4.30.

Three families derived from Cross 4.2.1A were selected and the plants of these three families (a-m En Sh/a-m-1 sh) were crossed onto their full-sibs (a-m-1 sh/a-m-1 sh) (sibcrosses, Cross 4.4.1A) and onto an unrelated a-m-1 sh/a-m-1 sh tester (testcrosses, Cross 4.4.1C). At the same time, the plants of these three families were selfed (Cross 4.4.1B). The frequencies of the loss events of the three kinds of crosses for

Table 4.35. Heritability of the "zero" state of En61138-3 in the testcross progeny of 83 1254 (Cross 4.2.1C)

Progeny (1983 ♂ number)	States of En61138-3[a] ℓ-1	m-1	s-1	Total % of loss events	% of "zero" in the total loss events	% of s-1 in the total loss events
Parent 82 1309/1110-1 ("zero") (Cross 4.2.1B)						
1254-1	0[b]	0	0	0	100	0
-2	-	-	1.0	1.0	0	100
-3	-	-	1.0	1.0	0	100
-4	-	-	1.0	1.0	0	100
-5	0	0	0	0	100	0
-7	-	-	1.0	1.0	0	100
-8	-	-	1.0	1.0	0	100
-9	0	0	0	0	100	0
-10	1.0	1.0	1.0	3.0	0	33.3
-11	-	-	4.0	4.0	0	100
-12	0	0	0	0	100	0
			average % of "zero" in the progeny = 36.4%			
			average % of "s-1" in the progeny = 63.3%			

[a]See Figure 3.2.

[b]See footnote of Table 4.30.

these three families are presented in Table 4.39. It is evident that the sibcrosses exhibit the greatest frequency of loss events in all three families (15.5%, 17.2%, and 22.5% of loss events for 82 1141-1142, 82 1143-1144, and 82 1115, respectively). This is followed by the selfed progeny that is the second higher type showing 11.8%, 12.8%, and 15.5% for 82 1141-1142, 82 1143-1144, and 82 1115 families, respectively.

Table 4.36. Heritability of the "zero" state of En61138-3 in the testcross progeny of 83 1255 (Cross 4.2.1C)

Progeny (1983 ♂ number)	States of En61138-3[a] m-1	s-1	Total % of loss events	% of "zero" in the total loss events	% of s-1 in the total loss events
Parent 82 1246/1118-4 ("zero") (Cross 4.2.1B)					
1255-1t	0.6[b]	2.4	3.0	0	80
-4	-	2.0	2.0	0	100
-5t	-	1.0	1.0	0	100
-6	-	4.0	4.0	0	100
-7	-	2.0	2.0	0	100
-10	-	2.0	2.0	0	100
-12	0	0	0	100	0
			average % of "zero" in the progeny = 14.3%		
			average % of "s-1" in the progeny = 82.9%		

[a] See Figure 3.2.

[b] See footnote of Table 4.30.

The lowest frequency of the loss events is the testcross group. It has only about one-half to one-third the amount of the frequency expressed with the sibcrosses or selfed progeny.

Among the three kinds of crosses, the frequency of loss event shows a significant difference between the sibcrosses and the testcrosses in all three families, but only the difference between selfed progeny and testcrosses is significant in 82 1141-1142 family by t-test. The results are presented in Table 4.40.

These observations reveal that some other factor(s) apart from the En of the a-m allele are included in either the male parents or the

Table 4.37. Heritability of the "zero" state of En61138-3 in the progeny of 83 1307 (Cross 4.2.1C)

Progeny (1983 ♂ number)	States of En61138-3[a]				Total % of loss events	% of zero in the total loss events	% of s-1 in the total loss events
	ℓ-1	m-2	s-1	s-2			
Parent 82 1256/1113-10 (zero) (Cross 4.2.1B)							
1307-1	-	-	0.5	0.5	1.0	0	50
-2	0[b]	0	0	0	0	100	0
-3	0.7	-	1.3	0	2.0	0	65
-4	0	0	0	0	0	100	0
-5	0	0	0	0	0	100	0
-8	-	-	1.0	-	1.0	0	100
-9	0	0	0	0	0	100	0
-10	0	0	0	0	0	100	0
			average % of "zero" in the progeny = 62.5%				
			average % of "s-1" in the progeny = 26.9%				

[a] See Figure 3.2.

[b] See footnote of Table 4.30.

Table 4.38. Heritability of the "zero" state of En61138-3 in the testcross progeny of 83 1308 (Cross 4.2.1C)

Progeny (1983 ♂ number)	States of En61138-3[a] ℓ-1	m-2	s-1	s-2	Total % of loss events	% of zero in the total loss events	% of s-1 in the total loss events
Parent 82 1252/1120-2t (zero) (Cross 4.2.1B)							
1308-1	-	-	1.0	-	1.0	0	100
-3	-	-	3.0	-	3.0	0	100
-4	0[b]	0	0	0	0	100	0
-5	-	-	1.0	-	1.0	0	100
-7t	0	0	0	0	0	100	0
-8	-	-	1.0	-	1.0	0	100
-9t	-	-	2.0	-	2.0	0	100
-11	-	-	1.0	-	1.0	0	100
-11t	0	0	0	0	0	100	0
			average % of "zero" in the progeny = 33.3%				
			average % of "s-1" in the progeny = 66.7%				

[a]See Figure 3.2.

[b]See footnote of Table 4.30.

Table 4.39. Mean and standard error of the frequency of En611383-ILE of three kinds of crosses for three families in 1982 (Crosses 4.4.1A, B, and C)

Family[a] \ Kinds of crosses	Sibcrosses (1)	Selfing (2)	Testcrosses (3)
82 1141-1142	15.52±1.35	11.81±2.64	5.45±1.32
82 1143-1144	17.20±4.79	12.83±3.43	9.36±2.32
82 1115	22.50±1.77	15.51±5.70	7.30±2.89

[a]Source of the families: (A) 82 1141-1142 are from ear 81 0919/1041-11 (Table 4.15, line 7; also see Table 4.19); (B) 82 1143-1144 are from ear 81 0921/1046-9 (Table 4.17, line 26; also see Table 4.20); (C) 82 1115 is derived from the kernels with colored shrunken sectors of the ear 81 0911/1032-8t (Table 4.13, line 19).

Table 4.40. Significant tests of paired t-values in different comparisons on the frequency of En61138-3-ILE among the crosses of three families in 1982

Family[a] \ Comparison	Sibcross-⊗	Sibcross-testcross	⊗-testcross
82 1141-1142	1.18^{ns}[b]	5.58**	3.07*
82 1143-1144	1.18^{ns}	2.69*	0.97^{ns}
82 1115	1.02^{ns}	6.16*	0.96^{ns}

[a]Source of families: See footnote of Table 4.39.

[b]ns = Nonsignificant.

**,*Significant at the 1% and 5% levels, respectively.

female parents of these three kinds of crosses to increase the frequency of the loss events.

Since the kernels derived from the selfing of a-m En Sh/a-m-1 sh (Cross 4.4.1B) have only one-third the chance to express the colored shrunken sectors in these tests compared with sibcrosses and testcrosses, the frequency of the loss event induced by En61138-3 in selfed progeny was actually three times the observed values obtained from the selfed ears. The reason has been discussed for this case in section 4.1.3.5. The new values for the selfed progeny have been calculated and are presented in Table 4.39, column (2).

There are no significant differences between selfed and testcross progeny in family 83 1143-1144 and in 83 1115, though the average value of the loss events in selfed progeny is about 1.5 to 2 times that in the testcross progeny of these two families. This result is caused by a greater variation within selfed and testcross progeny of these two families.

4.4.1.2. Tests of the effects of male and female parents on the frequency of En61138-3 loss event In order to further uncover factors influencing loss events, the male parents (a-m En Sh/a-m-1 sh) and the female parents (a-m-1 sh/a-m-1 sh) were selected, respectively, from the populations with a high frequency of loss events and a low frequency of loss events. Both of these populations were derived from the crosses of a-m-1 sh/a-m-1 sh tester and a-m En Sh/a-m-1 sh (En61138-3) plants (Cross 4.2.1A).

The diallel crosses among the parents derived from the populations with high or low frequency of loss event were made in 1982 as follows:

(1) H x H crosses (Cross 4.4.1D): Both the female parents (a-m-1 sh/a-m-1 sh) and male parents (a-m En Sh/a-m-1 sh), with and without loss sectors, were selected from ears 81 0911/1032-8t (Table 4.13, line 19), 81 0919/1041-11 (Table 4.15, line 7), and 81 0921/1046-9 (Table 4.17, line 20) with a high frequency of loss event. Male parents were crossed onto their full-sib female parents. The cross type is sibcrosses and the sources of the parents are the same as that in Cross 4.4.1A.

(2) H x L crosses (Cross 4.4.1E): The female parents (a-m-1 sh/a-m-1 sh) used in these experiments were derived from the same source as that for H x H with a high frequency of loss event (Cross 4.4.1D). Male parents a-m En Sh/a-m-1 sh were from ears with a low frequency of loss events [bulked sample of kernels with and without loss sectors from ears 81 0930/1031-11, 0902/1034-1 (Table 4.13, lines 12, 31), 81 0911/1035-11, 81 0903/1036-6, 81 0929/1037-10, 81 0906/1039-11, 81 0926/1040-10, 81 0925/1040-11 (Table 4.14, lines 4, 6, 21, 33, 50, 51), 81 0909/1043-4, 81 0924/1043-6, 81 0923/1044-5t, 81 0925/1044-11, 81 0923/1044-11t (Table 4.16, lines 4, 6, 15, 18, 19), 81 0917/1045-6, 81 0947/1046-5 (Table 4.17, lines 7, 24), 81 0922/1047-3, 81 0947/1047-6, 81 0927/1047-7t, 81 0946/1047-8t, and 81 0950/1048-1 (Table 4.18, lines 2, 5, 10, 14, 15)].

(3) L x H crosses (Cross 4.4.1F): Female parents (a-m-1 sh/a-m-1 sh) were selected from ears 81 0902/1034-1, 81 0903/1036-6 (Table 4.13, line 31 and Table 4.14, line 6) and a bulked sample with a low frequency of loss event [81 0930/1031-11 (Table 4.13, line 12), 81 0929/1037-10, 81 0926/1040-10, 81 0925/1040-11 (Table 4.14, lines 21, 50, 51), 81 0924/1043-6, 81 0923/1044-5t, 81 0925/1044-11 (Table 4.16, lines 6, 15, 18), 81 0922/1047-3, 81 0947/1047-6, 81 0927/1047-7t, and 81 0946/1047-8t (Table 4.18, lines 2, 5, 10, 14)]. Male parents (a-m En Sh/a-m-1 sh) were derived from kernels with and without loss sectors from ears 81 0921/1032-8t, 81 0911/1032-8t (Table 4.13, lines 18, 19), 81 0919/1041-11 (Table 4.15, line 7), and 81 0921/1046-9 (Table 4.17, line 20) which expressed a high frequency of loss events.

(4) L x L crosses (Cross 4.4.1G): Female parents (a-m-1 sh/a-m-1 sh) were from ears with a low frequency of loss event (81 0902/1034-1 and 81 0903/1036-6 (Table 4.13, line 31; Table 4.14, line 6)). Part of the male parents were from the same source as that used in H x L crosses (Cross 4.4.1E), the remaining are from ear 81 0902/1034-3 (Table 4.13, line 32).

At the same time, the male parents in each kind of cross were crossed onto an unrelated a-m-1 sh/a-m-1 sh tester as a control (Cross 4.4.1H). The pooled frequencies of the loss event in each class of crosses were compared with each other and compared with the data obtained from test-crosses (Cross 4.4.1H). The results are shown in Table 4.41. These

Table 4.41. Comparison of the level of En61138-3-ILE in the diallel crossed by t-test

♂[a] \ ♀[a]	High (1)	Low (2)	Effect of ♀ (1)-(2)	Unrelated a-m-l sh/ a-m-l sh (♀) tester (check)
High (3)	21.15±2.55 ——	13.4±2.11	+7.75(+36.64%)	7.5±0.6
Low (4)	9.94±1.30 ——	7.81±0.85	+2.13(+21.43%)	3.5±0.7
Effect of ♂ (3)-(4)	+11.21 (+53%)	+5.59 (+41.72%)		+4 (+53.3%)

[a]Sources of female and male parents are described in the text.

——Shows no significant difference on the level of En61138-3-ILE between two crosses.

----Shows highly significant at 1% level on the level of En61138-3-ILE between two crosses.

results indicate that the effect of female parents (a-m-l sh/a-m-l sh) on the level of En61138-3-ILE is not significant irrespective of the source populations. There was a 36.6% reduction of the frequency of loss events from HxH to LxH crosses, and a 21.4% reduction of the frequency of loss events from HxL to LxL crosses. However, there were highly significant differences in the level of En61138-3-ILE between HxH vs. HxL (53%) and LxH vs. LxL crosses (41.7%). The control also showed 53% significant reduction in the frequency of loss

event from high (H) loss male parents to low (L) loss male parents, but the actual frequencies of loss events were much lower than that in the diallel crosses (Table 4.41).

Additionally, the frequency of En61138-3-ILE in the progeny from HxH crosses was significantly higher than that from LxL crosses. The difference was not significant between HxL and LxH crosses, though the frequency of loss event is slightly higher in LxH than that in HxL crosses (Table 4.41).

From the significant change of the frequency of loss events arising from the male parent, it is evident that the factors causing a difference on the level of En61138-3-ILE are mainly from the male parents and these factors are independent of the En of the a-m En allele in the genome.

The action of these factors on the level of En61138-3 was additive. The greater the number of factors accumulating in the progenies led to an increase of En61138-3-ILE (Table 4.41).

4.4.1.3. The continuity of the effect of male and female parents on the level of En61138-3-ILE To assess whether the factors causing the different levels of En61138-3 will be transmitted to the next generation, round spotted kernels (En61138-3) were selected from those four combinations of diallel crosses (Cross 4.4.1D, 4.4.1E, 4.4.1F, and 4.4.1G) were crossed onto the a-m-1 sh/a-m-1 sh tester in 1983,

a-m-1 sh/a-m-1 sh x a-m En Sh/a-m-1 sh . Cross 4.4.1I

The results of Cross 4.4.1I are presented in Table 4.42. It was found that the frequencies of En61138-3 loss events in the testcross progeny retain the same trend as that in the diallel crosses shown in Table 4.41. The different levels of En61138-3-ILE mainly depend on the male parents.

Table 4.42. Comparisons of the level of En61138-3-ILE in testcross progeny derived from the crosses of the plants selected from the progeny of diallel crosses and the a-m-1 sh/ a-m-1 sh tester in 1983

	High	Low	Effect of ♀ parents (high-low)
High	3.87±1.23 ——	2.79±0.81	-1.08(-27.9%)
	¦ ╲ ¦	¦	
Low	1.05±0.34 ——	0.88±0.26	-0.17(-16.2%)
Effect of ♂ parents	-2.82 (-72.9%)	-1.91 (-68.5%)	

——No significant difference between two crosses by t-test.

----Highly significant difference at the 1% level between two crosses by t-test.

In addition, the factors increasing the frequency of En61138-3 in the male parents segregate independently to the progeny.

The actual frequencies of En61138-3-ILE were much lower in the testcross progeny in 1983 than that of the crosses among diallel progeny in 1982 and also lower than that of the testcross progeny with respect to the male parents in 1982 (Table 4.41 vs. Table 4.42).

4.4.1.4. Tests of the possibility of the modifiers in testcross, selfing, and sibcross populations In studies of the effect of the three kinds of crosses on the level of the En61138-3 related loss event, it has been proposed that there were some factors in the genome affecting the level of the loss event (section 4.4.1.1 and 4.4.1.2).

Now it is assumed that a number of modifiers exist in the population of En61138-3 that are segregating independently and function in increasing the frequency of the loss event. It is also proposed that the frequency of the loss event is proportional to the frequency of modifiers in the population in addition to the action of En61138-3.

On the assumption of the modifiers being mainly from male parents (a-m En Sh/a-m-1 sh) and no modifiers present in the recurrent female parents (unrelated a-m-1 sh/a-m-1 sh tester), it would be expected that the total frequencies of the modifiers in the progeny will decrease to a half of that in their parental population after every testcross irrespective of the original frequencies of the modifiers. However, the total frequencies of the modifiers in the progeny will be maintained at the same level as their parental population when selfing or sibcross was executed. Therefore, the ratio of the total frequencies of the modifiers in testcross, selfing, and sibcross population is 1:2:2 in the same generation. An example is given as follows:

Suppose there is one modifier (mod) in the population of En61138-3 in 1980, and it is in heterozygote constitution (mod/-). Sibcrosses, selfing, and testcrosses were made in summer of 1980, and assuming no modifiers are in the female parents in testcross, then the total frequencies of modifiers in these three populations are shown as in the following:

Year	Crosses and genotypes of progeny	Freq. of mod
(1) 1980	mod/-	1/2
(2) 1980 summer	sibcross: mod/- x mod/- → mod/mod, 2 mod/-, -/-	1/2
	selfing: mod/- ⊗ → mod/mod, 2 mod/-, -/-	1/2
	testcross: -/- x mod/- → 2 mod/-, 2 -/-	1/4
	select kernels from testcross progeny for 1981 experiments ↙	
(3) 1981	mod/-, -/-	1/4
(4) 1981 summer	sibcross: mod/- x mod/- → mod/mod, 2 mod/-, -/- mod/- x -/- → 2 mod/-, 2 -/- -/- x -/- → 4 -/-	1/4
	selfing: mod/- ⊗ → mod/mod, 2 mod/-, -/- -/- ⊗ → 4 -/-	1/4
	testcross: -/- x mod/- → 2 mod/-, 2 -/- -/- x -/- → 4 -/-	1/8

From this example, it is seen that the total frequency of modifiers in testcross population of 1981 is 1/4, and this frequency will be kept at the same level through selfing or sibcross in 1981 summer. Yet only half of this frequency (1/8) will be obtained through the testcross series in the 1981 summer. As a result, the frequency of the loss event should be given in sibcross, selfing, and testcross populations with the ratio of 2:2:1. This ratio will be kept constant if no other forces, such as selection, changes the frequency of the modifiers in the population.

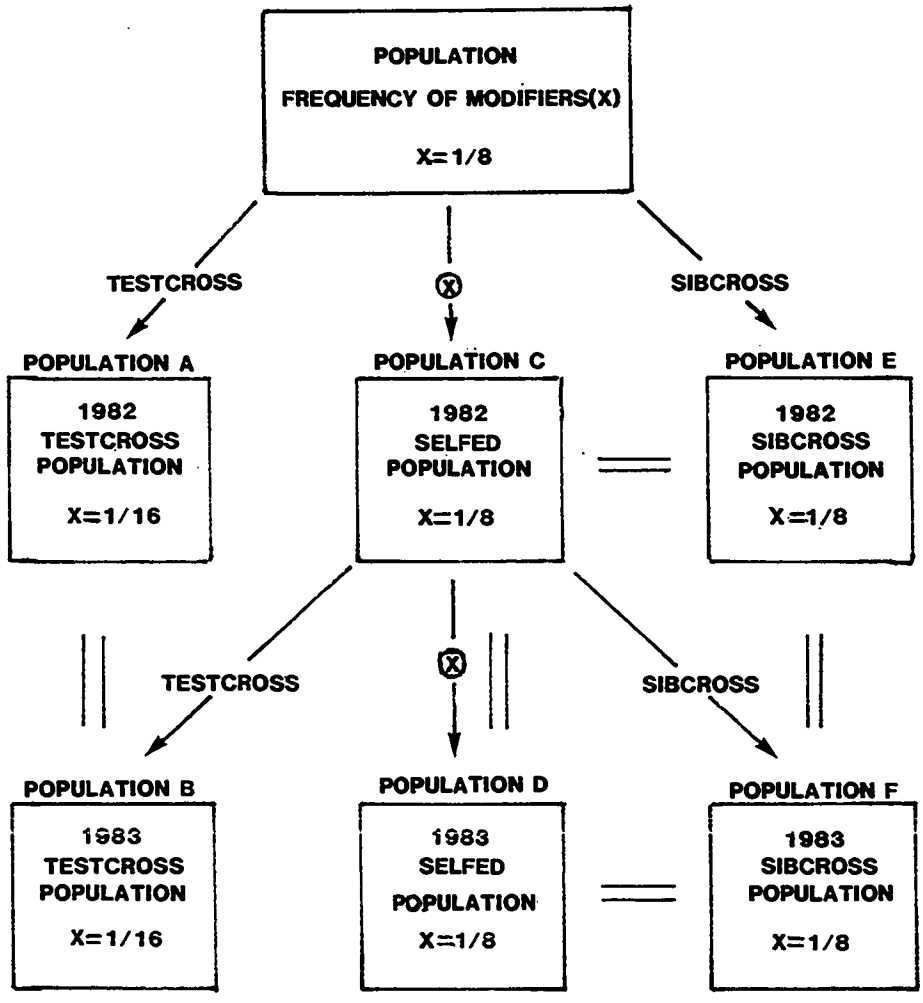
According to this hypothesis, the frequency of modifiers can be maintained by selfing or sibcross from one generation to the next generation. Therefore, the average frequency of the loss events induced by En61138-3 of a selfed or a sibcrossed population in a generation should be equal to that of the selfed or sibcrossed population of the previous generation. A similar deduction can be applied to the testcross population. The average frequency of the loss event in a testcross population can be kept at the same level as its parental population through one selfing or sibcrossing. A hypothetical illustration is shown in Figure 4.21 where it is assumed that the frequency of the modifiers in one population is 1/8 in 1982. This 1/8 will be kept constant to 1983 by selfing or sibcrossing the 1982 selfed or sibcrossed population. The frequency of the modifiers in the testcross population of 1983 in which its male parents are derived from the selfed or sibcrossed plants of 1982 should be the same as that of 1982 testcross population. Therefore, the average frequencies of loss events in each selfed or sibcross population of 1982 and 1983 are expected to be the same (populations: C=D=E=F of Figure 4.21). The same average frequency of the loss event is expected to be found in 1982 and 1983 testcross population (population A = population B of Figure 4.21).

To test these possibilities, the data obtained from three families of 1982 and four families of 1983 were analyzed statistically by analysis of variance and t-test. The model for the analysis of variance for testing the loss frequency of sibcross, selfed, and testcross populations with 2:2:1 ratio is as follows:

Figure 4.21. A diagram illustrating the relations of the changes of the frequency of the modifiers in different populations

x represents the total frequencies of the modifiers in the population

= represents the average frequency of En61138-3-ILE between two populations is approximately equal


POPULATION
FREQUENCY OF MODIFIERS(X)
X=1/8
TESTCROSS
⊗
SIBCROSS
POPULATION A
1982
TESTCROSS
POPULATION
X=1/16
POPULATION C
1982
SELFED
POPULATION
X=1/8
POPULATION E
1982
SIBCROSS
POPULATION
X=1/8
TESTCROSS
⊗
SIBCROSS
POPULATION B
1983
TESTCROSS
POPULATION
X=1/16
POPULATION D
1983
SELFED
POPULATION
X=1/8
POPULATION F
1983
SIBCROSS
POPULATION
X=1/8

$$Y_{ijk} = \mu + F_i + P_j + (FP)_{ij} + e_{ijk}$$

where

μ = grand mean;

F_i = i^{th} family effect;

P_j = j^{th} population effect;

$(FP)_{ij}$ = interaction effect between i^{th} family and j^{th} population;

e_{ijk} = error;

$i = 1, 2, 3, \ldots \ell$;

$j = 1, 2, 3, \ldots m$; and

$k = 1, 2, 3, \ldots n$.

Hypotheses for testing the sibcross, selfing, and testcross population with the ratio 2:2:1 are:

(1) Ho: $\bar{Y}\cdot 1\cdot - \bar{Y}\cdot 2\cdot = 0$

(2) Ho: $(\bar{Y}\cdot 1\cdot + \bar{Y}\cdot 2\cdot)/2 - 2\bar{Y}\cdot 3\cdot = 0$.

t-test for hypothesis (1) is:

$$t = \frac{\bar{Y}\cdot 1\cdot - \bar{Y}\cdot 2\cdot}{\sqrt{\frac{MSE}{n_1} + \frac{MSE}{n_2}}}$$

and t-test for hypothesis (2) is:

$$t = \frac{[(\bar{Y}\cdot 1\cdot + \bar{Y}\cdot 2\cdot)/2 - 2\bar{Y}\cdot 3\cdot]}{\sqrt{S_x^2}}$$

where

$\bar{Y}\cdot 1\cdot$ = mean of the frequency of En61138-3-ILE of sibcross population;

$\bar{Y}\cdot 2\cdot$ = mean of the frequency of En61138-3-ILE of selfing population;

$\bar{Y}\cdot 3\cdot$ = mean of the frequency of En61138-3-ILE of testcross population; and

$$S_x^2 = \text{var}[(\frac{\bar{Y}\cdot 1\cdot + \bar{Y}\cdot 2\cdot}{2}) - 2\bar{Y}\cdot 3\cdot]$$

$$= \text{MSE}(\frac{1}{4}\cdot\frac{1}{\ell n_1} + \frac{1}{4}\cdot\frac{1}{\ell n_2} + 4\cdot\frac{1}{\ell n_3})$$

where

ℓ = no. of families and

n_j = no. of observations in j^{th} population.

The means of the frequency of En61138-3-ILE in three different populations in 1982 and 1983 are presented in Table 4.43. Comparing the families of each population in 1982 and 1983, the trend of the frequency of the loss event seemingly depends on the frequency of the loss event of the parent of each family. Parents with high loss frequency yield high loss frequency in their progeny. This result is coincident with that obtained in section 4.2.1.

A test is made to examine the possibility that the average frequencies of En61138-3 are the same among four populations (Figure 4.21, populations C=D=E=F) and the frequency of En61138-3-ILE of testcross population of 1982 is approximately equal to that of testcross population of 1983 (Figure 4.21, populations A=B). The results of t-test are shown in Table 4.44. It showed that no significant differences of the level of En61138-3-ILE were found among the four populations (populations C, D, E, F of Figure 4.21). The difference of the level of En61138-3-ILE between 1982 and testcross populations was also not significant.

The results of analysis of variance for testing the frequency of En61138-3-ILE in sibcross, selfing, and testcross population with the

Table 4.43. Mean of the frequencies of En61138-3-ILE in sibcross, selfing, and testcross populations of the families in 1982 and 1983

Family[a]	Sibcross[b]	Selfing[b]	Testcross[b]
82 1141-1142	15.52±1.35	11.81±2.64	5.45±1.32
82 1143-1144	17.20±4.79	12.83±3.43	9.36±2.32
82 1115	22.50±1.77	15.51±5.70	7.30±2.89
Pooled $\bar{x}\pm s_{\bar{x}}$	17.5±1.6	12.9±1.9	7.0±1.1
83 1334	14.5±1.9	15±5.5	8.5±6.5
83 1339	13	15	5
83 1342	17.7±8.4	10±5.3	4.3±2.3
83 1343	27.5±8.5	27±2.4	3.5±3.5
Pooled $\bar{x}\pm s_{\bar{x}}$	17.9±3.1	15.9±4.8	5.9±2.6

[a]Families of 1982 were derived from the testcross progeny of a-m-1 sh/a-m-1 sh x En61138-3/a-m-1 sh (Cross 4.2.1A). Sources of these families are shown in the footnote of Table 4.39. Families of 1983 were derived from the selfed progeny of a-m En Sh/a-m-1 sh parents of 1982. Sources of these four families are as follows: 83 1334 is from the selfed ear of 82 1143-6 with 11.1% loss frequency; 83 1339 is from the selfed ear of 82 1149-1 with 10.8% loss frequency; 83 1342 is from the selfed ear of 82 1153-1 with 7.0% loss frequency; and 83 1343 is from the selfed ear of 82 1115-3 with 66.6% loss frequency.

[b]Different populations are derived from the Cross 4.4.1A, 4.4.1B, and 4.4.1C, respectively, in 1982 (see section 4.4.1.1). Similar procedures were carried out in 1983 to produce populations.

ratio of 2:2:1 in 1982 and in 1983 are shown in Table 4.45. Highly significant difference was found among the different populations in 1982. However, the frequency of En61138-3-ILE among the populations in 1983 was not significantly different, though the mean of the frequency of

Table 4.44. Means of the En61138-3-ILE of sibcross, selfing, and testcross populations in 1982 and 1983 and test of the difference between two generations in each cross by t-test

Generation / Population	1982[a]	1983[b]	t-value
Sibcross	17.5±1.6	17.9±3.1	.124[ns c]
	.700[ns] 1.43[ns] .734[ns] .158[ns]		
Selfing	12.9±1.9	15.9±4.8	.655[ns]
Testcross	7.0±1.1	5.9±2.6	.09[ns]

[a]Pooled data of three families of 1982 (Table 4.43).

[b]Pooled data of four families of 1983 (Table 4.43).

[c]ns = Nonsignificant.

sibcross or selfed population seems higher than that of testcross population. This is because the experimental error is greater in 1983 (see Tables 4.43 and 4.45).

No difference among families and no significant interaction effect between family and population were found. This result indicates that the responses of the frequency of En61138-3-ILE to different kinds of crosses among families have the same trend. Combined analysis of variance (combined data of 1982 and 1983) is shown in Table 4.46. It provides support for the same results obtained in Table 4.45.

To test hypothesis (1) ($\bar{Y}\cdot 1\cdot - \bar{Y}\cdot 2\cdot = 0$) and hypothesis (2) [$(\bar{Y}\cdot 1\cdot + \bar{Y}\cdot 2\cdot)/2 - 2\bar{Y}\cdot 3\cdot = 0$], a t-test is made and the t-values are shown in Table 4.47.

Table 4.45. Analysis of variance on the frequency of En61138-3-ILE for families and populations in 1982 and in 1983

Year	Source of variation	df	Mean square
1982	Family (F)	2	58.9^{ns}[a]
	Population (P)	2	390.5**
	F x P	4	13.3^{ns}
	Error	33	35.6
1983	Family (F)	3	100.5^{ns}
	Population (P)	2	413.4^{ns}
	F x P	6	59.2^{ns}
	Error	18	158.8

[a] ns = Nonsignificant.

** Significant at .01 by F-test.

Table 4.46. Combined analysis of variance on the frequency of En61138-3-ILE for families and populations of 1982 and 1983

Source of variation	df	Mean square
Family (F)	6	71.6^{ns}[a]
Population (P)	2	77.9**
F x P	12	38.2^{ns}
Error	51	79.3

[a] ns = Nonsignificant.

** Significant at .01 by F-test.

Table 4.47. t-values on the comparisons of sibcross, selfing, and testcross populations of 1982, 1983, and the combined data of 1982 and 1983

Comparison / Year	Sibcross-selfing ($\bar{Y}\cdot 1\cdot - \bar{Y}\cdot 2\cdot$)	[(Sibcross+selfing)/2 -2testcross)] [($\bar{Y}\cdot 1\cdot + \bar{Y}\cdot 2\cdot$)/2 $-2\bar{Y}\cdot 3\cdot$]
1982	2.028^{ns}[a]	0.61^{ns}
1983	0.35^{ns}	0.59^{ns}
Combined data	1.75^{ns}	1.51^{ns}

[a] ns = Nonsignificant by t-test.

The results showed that the frequencies of En61138-3-ILE are the same between sibcross and selfed populations. Half of the frequency of En61138-3-ILE of sibcross or selfed population is equal to that of testcross population.

From all of these results, it is evident that the hypothesis on condition and frequency of modifiers influencing the En61138-3-ILE is acceptable under these assumptions:

(1) a number of modifiers exist in the En61138-3 populations;

(2) these modifiers increase the frequency of the loss event in proportion to the frequency of the modifiers in that population;

(3) the frequency of the modifiers can be maintained by selfing or sibcross, but will be reduced to half of that of its parental population if no other force affects the frequency of the modifiers in the population;

(4) these modifiers segregate independently; and

(5) there is no dominant gene action in these modifiers.

4.4.2. Effect of main stalk and tiller

4.4.2.1. Comparison of the difference between main stalk and tiller on the level of En61138-3-ILE In an attempt to determine the effect of main stalk and the tiller of the same plant on the frequency of En61138-3-ILE, two families (82 1141-1142 and 82 1143-1144) derived from the kernels with and without loss sectors were involved in this experiment. One tiller for each plant was saved. The male gametes of the main stalk and tiller of the same plant containing the En61138-3 allele were crossed onto a-m-1 sh/a-m-1 sh tester plants, respectively, in 1982. At the same time, main stalk ear and tiller ear were selfed. The crosses are shown as follows:

a-m-1 sh/a-m-1 sh x a-m En Sh/a-m-1 sh (main stalk) Cross 4.4.2A

↓

⊗ Cross 4.4.2C

a-m-1 sh/a-m-1 sh x a-m En Sh/a-m-1 sh (tiller) Cross 4.4.2B

↓

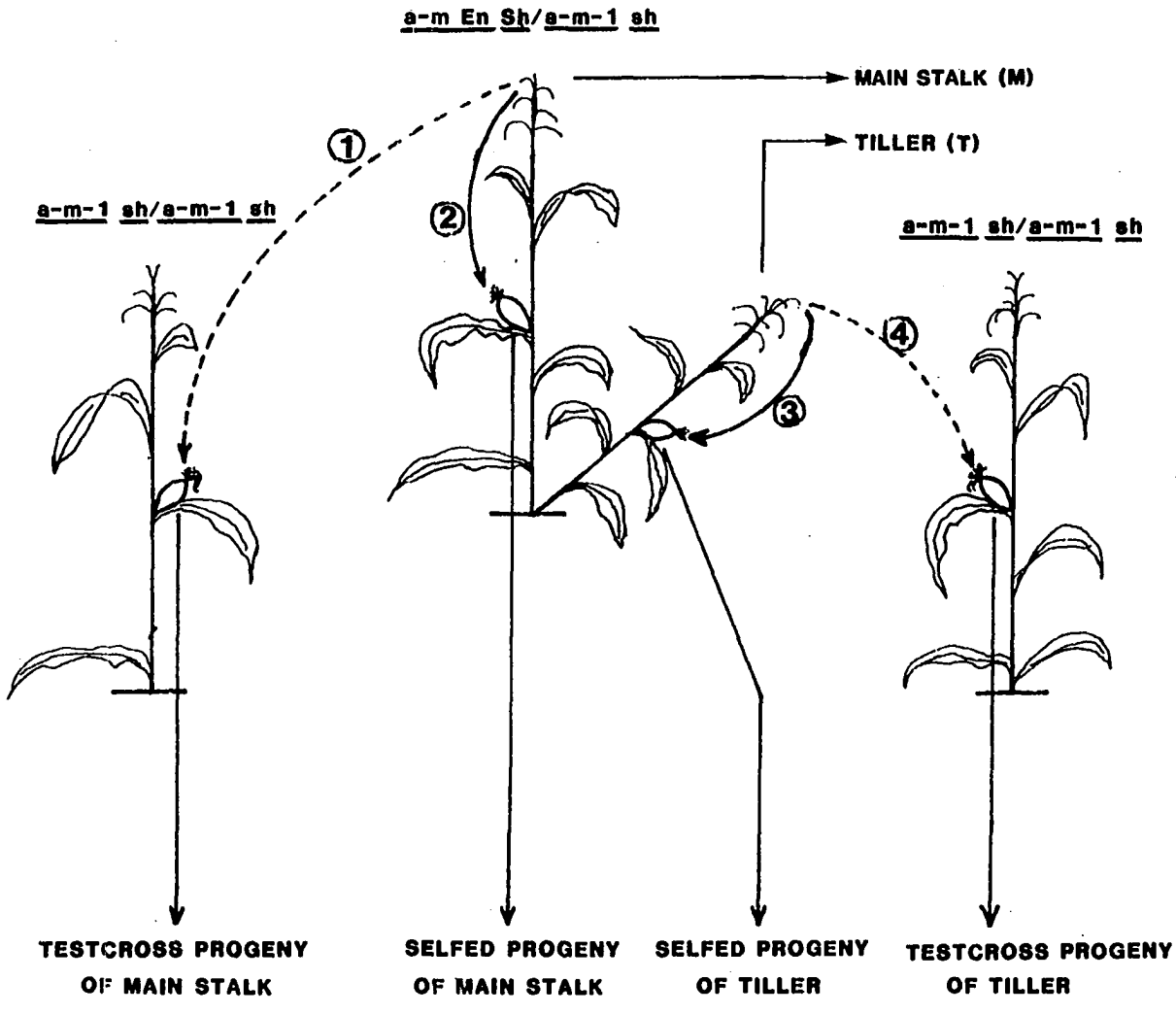
⊗ Cross 4.4.2D

The procedures outlining these crosses are graphed in Figure 4.22.

Fourteen and 20 pairs of comparisons in testcross and selfed progeny, respectively, were available for comparing the difference between main stalk and tiller on the level of En61138-3-ILE. Nested design was used to ascertain the effect of main stalk and tiller of the same plants on the frequency of En61138-3-ILE.

Figure 4.22. Diagrammatic illustration of a main stalk and tiller arising from a single kernel. Testcross progeny and selfed progeny of main stalk and tiller are also shown in this diagram.

① = Cross 4.4.2A, ② = Cross 4.4.2C, ③ = Cross 4.4.2D, ④ = Cross 4.4.2B


a-m En Sh/a-m-1 sh
MAIN STALK (M)
TILLER (T)
a-m-1 sh/a-m-1 sh
a-m-1 sh/a-m-1 sh
1
2
3
4
TESTCROSS PROGENY OF MAIN STALK
SELFED PROGENY OF MAIN STALK
SELFED PROGENY OF TILLER
TESTCROSS PROGENY OF TILLER

The model for nested design was as follows:

$$Y_{ijk\ell} = \mu + F_i + K(F)_{ij} + S(K/F)_{ijk} + e_{ijk\ell}$$

where

μ = grand mean;

F_i = effect of i^{th} family; i=1,2;

$K(F)_{ij}$ = effect of j^{th} kind of kernels (with loss sectors vs. without loss sectors) within i^{th} family; j=1,2;

$S(K/F)_{ijk}$ = effect of k^{th} plant part (main stalk vs. tiller) within j^{th} kernel within i^{th} family; k=1,2; and

$e_{ijk\ell}$ = error; ℓ=1,2,3,...ℓ (no. of observations).

The results of analysis of variance indicated that there is no significant difference on the frequency of the loss event between the main stalk and the tiller both in testcross and selfed progeny (Table 4.48).

4.4.2.2. Contingency χ^2-test on the level of En61138-3-ILE in the male gametes from main stalk and tiller on individual plant basis

4.4.2.2.1. Effect of main stalk and tiller on the level of En61138-3-ILE in 1982 To assess the consistency of the frequency of the loss event between main stalk and tiller of the same plant, the contingency χ^2-test was used to compare the level of En61138-3-ILE in main stalk and tiller with the same data of section 4.4.2.1.

In testcross progeny, the χ^2-test showed that the main stalk and tiller of the same plant expresses the same level of En61138-3-ILE. Eleven out of 14 comparisons showed an approximately equal frequency of loss events between main stalk and tiller (Table 4.49), while there were two comparisons which showed significantly higher loss frequency

Table 4.48. Analysis of variance for the effects of main stem and tiller of the same individual plant on the occurrence of sectors in two families from the crosses of round spotted kernels on colorless background with colored shrunken sector and without colored shrunken sector and a-m-1 sh/a-m-1 sh tester and selfed progeny of the plants containing En61138-3 alleles (a-m En Sh/a-m-1 Sh)

Source of variation	df	MS	
		outcrossing	selfing
Families	1	0.019[ns][a]	0.015[ns]
Kernels (Family)[b]	2	0.006[ns]	0.014[ns]
Stems (Kernel/Family)[c]	4	0.007[ns]	0.004[ns]
Error	32	0.004	
	16		0.008

[a]ns=Nonsignificant by F-test.

[b]Kernels with loss sectors vs. without loss sectors within family.

[c]Main stalk vs. tiller within kernel within family.

in main stalk than that in the tiller (Table 4.49, lines 21-22, 27-28). One other comparison showed a significantly lower frequency of loss event in the main stalk than that in the tiller (Table 4.49, line 9-10).

In the selfed progeny, most of the cases indicated that the level of En61138-3-ILE was similar between the main stalk and tiller (Table 4.50). Six out of 20 comparisons showed a significantly higher or lower frequency of the loss events in the main stalk compared with the tiller. Three of these six comparisons had a significantly higher loss frequency in the main stalk (Table 4.50, lines 21-22, 23-24, 31-32), and the other three had a significantly lower loss frequency in the main stalk

Table 4.49. Comparison of the frequency of the loss events resulting from the crosses of a-m-1 sh/a-m-1 sh tester and the main stalk (a-m En Sh/a-m-1 sh) and that of a-m-1 sh/a-m-1 sh tester and the tiller (a-m En Sh/a-m-1 sh)[a] (Cross 4.4.2A and Cross 4.4.2B)

Male plant pedigree no.	Round: Nonspotted colored	Round: Spotted colorless: No sector	Round: Spotted colorless: Large sector	Round: Spotted colorless: Med. sector	Round: Spotted colorless: Small sector	% of sector	Shrunken nonspotted colored	Contingency of χ^2 between main and tiller ear on the same plant
1. 1141-2M[b]		43			5	10.42	56	0.0007
2. -2T		24			3	11.11	39	
3. -8M		245	2		19	7.89	264	0.3230
4. -8T		21			1	4.55	24	
5. -9M		112	1		2	2.61	95	0.7340
6. -9T		122			6	4.69	154	
7. 1142-1M[b]		43			3	6.52	57	1.3462
8. -1T	1	110			3	2.65	121	
9. -5M		141		1		0.70	143	6.193*
10. -5T		158	1		9	5.95	171	
11. -7M		210	1	2	7	4.55	258	3.1621
12. -7T		102			1	0.97	115	
13. -8M		38			3	7.32	55	2.8200
14. -8T		142			3	2.07	143	
15. 1143-3M[c]	8	96			4	4.00	66	2.8494
16. -3T		20			3	13.04	30	
17. -4M		48		1	6	12.73	53	2.2396

[a]Data is from Tables 4.19 and 4.20.

[b]82 1141, 1142 seed source from 81 0919/1041-11 with and without sector, respectively (Table 4.15, line 7; also see Table 4.19).

[c]82 1143, 1144 seed source from 81 0921/1046-9 with and without sector, respectively (Table 4.17, line 26; also see Table 4.20).

Table 4.50. Continued

Male plant pedigree no.	Round					% of sector	Shrunken nonspotted colored	Contingency of χ^2 between main and tiller ear on the same plant
	Nonspotted colored	Spotted colorless						
		No sector	Large sector	Med. sector	Small sector			
18. 1143-4T		71			4	5.33	86	
19. -5M		137			9	6.16	144	1.4718
20. -5T		115			13	10.16	128	
21. -6M		115		1	21	16.06	99	16.1357**
22. -6T		159			5	3.05	202	
23. 1144-2M[c]		144			16	10.00	133	0.7044
24. -2T		105			8	7.08	144	
25. -3M		33			1	2.94	33	0.1041
26. -3T		163			7	4.12	166	
27. -5M	1	56	1		6	11.11	67	5.8055*
28. -5T		94			2	2.08	132	

compared with their corresponding tiller (Table 4.50, lines 9-10, 13-14, 17-18).

Comparing the contingency χ^2-test results of testcross and selfed progeny, only one pair of comparisons has significant χ^2-values in both the testcross and selfed progeny (82 1142-5M vs. 82 1142-5T, Table 4.49, lines 9-10 and Table 4.50, lines 17-18). The frequency of the loss event was higher in the tiller than that in the main stalk of the same plant.

Both of the results in testcross and selfed progeny by contingency χ^2-test showed that the effect of main stalk and tiller of the same plant is not significant. The same result was obtained by an analysis of variance (Table 4.48).

4.4.2.2.2. Test of the contingency of the exceptional comparisons of the main stalk vs. tiller of the same plant in 1982 Following the contengency χ^2-test for comparing the effect of main stalk and tiller of the same plant on the frequency of the loss event in the testcross progeny of 1982, the round spotted kernels with and without loss sectors on colorless background were selected from the three exceptional pairs of comparisons (82 1142-5M vs. 1142-5T, 82 1143-6M vs. 1143-6T, and 83 1144-5M vs. 1144-5T) (Table 4.49, lines 9-10, 21-22, 27-28). Plants derived from these kernels were crossed onto the a-m-1 sh/a-m-1 sh tester (Cross 4.4.2E) to determine whether the difference on the level of the loss event between the main stalk and tiller of the same plant in each exceptional pair of comparisons is heritable from 1982 to 1983. The diagrams illustrating the procedures of crosses and comparisons for each exceptional pair of comparisons and their progeny are shown in Figures

Table 4.50. Comparisons of the frequency of En61138-3 resulting from the selfed progeny of the main stalk and tiller of the same plant containing the genotype, a-m En Sh/a-m-1 sh (Crosses 4.4.2C and 4.4.2D)

	Selfing[a] (1982)	Round spotted[b] No loss	Round spotted[b] Loss	% of loss sector	χ^2-value
1.	1141-1M	313	26	7.67	0.5794
2.	-1T	190	12	5.94	
3.	-2M	204	5	2.39	1.1302
4.	-2T	263	3	1.13	
5.	-5M	370	24	6.09	0.6064
6.	-5T	501	40	7.39	
7.	-7M	302	19	5.92	2.9257
8.	-7T	222	24	9.76	
9.	-9M	281	17	5.70	8.6660**
10.	-9T	270	39	12.62	
11.	1142-1M	155	8	4.90	1.7175
12.	-1T	612	18	2.86	
13.	-2M	254	11	4.15	8.5248**
14.	-2T	296	35	10.57	
15.	-4M	242	14	5.47	0.2837
16.	-4T	345	24	6.50	
17.	-5M	243	3	1.22	4.1480*
18.	-5T	566	23	3.90	
19.	-8M	197	4	1.99	0.4637
20.	-8T	190	6	3.06	

[a]Seed source is the same as in Table 4.46.

[b]The shrunken nonspotted colored kernels are not listed but appear approximately one-fourth in all the selfed progeny.

**,*Significant at .01 and .05, respectively.

Table 4.50. Continued

Selfing (1982)	Round spotted		% of loss sector	χ^2-value
	No loss	Loss		
21. 1143-1M	176	14	7.37	4.3138*
22. -1T	109	2	1.80	
23. -3M	289	22	7.07	6.2315*
24. -3T	241	6	2.43	
25. -4M	312	8	2.50	3.3737
26. -4T	303	2	0.66	
27. -5M	254	5	1.93	0.0637
28. -5T	241	4	1.63	
29. -6M	182	7	3.70	0.0868
30. -6T	155	7	4.32	
31. 1144-1M	194	7	3.48	5.4099*
32. -1T	298	2	0.67	
33. -2M	284	5	1.73	0.8464
34. -2T	232	7	2.93	
35. -3M	248	8	3.13	1.3936
36. -3T	351	6	1.68	
37. -5M	347	38	9.87	3.0208
38. -5T	98	4	3.92	
39. -6M	288	27	8.57	3.0613
40. -6T	184	8	4.17	

4.23, 4.24, and 4.25, respectively. The results of the contingency χ^2-test presented in Table 4.51 showed that all the frequencies of the loss event induced by En61138-3 were not significantly different between the main stalk and tiller of the same plant derived from two exceptional comparisons [four families (83 1319x-1319y, 1320-1321, 1325-1326, and 1327x-1327y)]. There was one exceptional comparison (82 1143-6M vs. 1143-6T) from which five of six comparisons showed significantly higher loss frequency in the tiller than that of the main stalk in 83 1322 progeny ears (Table 4.51, lines 27-36). However, these results are contrary to the results from their corresponding parents (82 1143-6M vs. 1143-6T, Table 4.49, lines 21-22) in which the main stalk showed significantly higher frequency of loss event than that of tiller.

The results from the analysis of variance on the frequency of the loss events occurring on the main stalk and tiller of the same plant showed that no significant difference exists between main stalk and tiller of the same parental plant in Cross 4.4.2E in these three exceptional pairs (Table 4.52, line 1), and it was also found that no significant difference exists between the main stalk and the tiller of the same plant within each family (progeny of Cross 4.4.2E) (Table 4.52, line 3).

According to the conclusions presented in section 4.4.1, the 83 1322 parent had the highest frequency of loss event (16%) among all parents of these three exceptional comparisons, indicating that it probably contained a large number of modifiers. These modifiers segregate independently and are distributed into its progeny; therefore, the individual plants within these progeny families should show a great variation on

Figure 4.23. An example diagrammed to illustrate the procedures of the crosses in a comparison of the frequency of En61138-3-ILE derived from a pair of exceptional comparisons of main stalk and tiller (82 1142-5M vs. 1142-5T). Also see Table 4.51, lines 1-2, 3-4, 9-10, 13-14. All females are a-m-1 sh/a-m-1 sh tester and all males are a-m En Sh/a-m-1 sh.

1=Cross 4.4.2A
2=Cross 4.4.2B
3=Cross 4.4.2E
(+)=Kernels with loss sectors were selected
(-)=Kernels without loss sectors were selected
(a)=a pair of comparisons

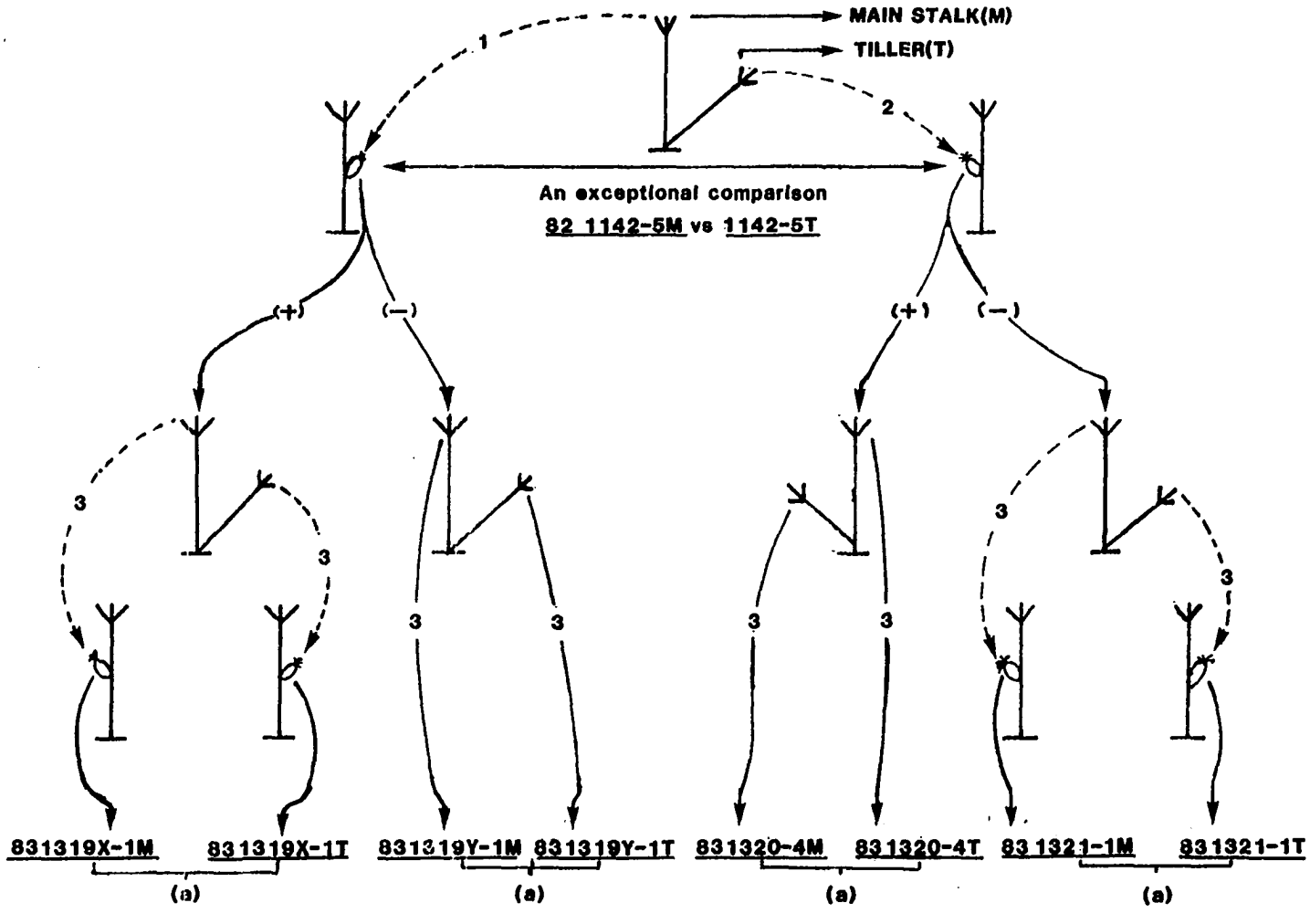

MAIN STALK(M)
TILLER(T)
1
2
An exceptional comparison
82 1142-5M vs 1142-5T
(+)
(−)
(+)
(−)
3
3
3
3
3
3
831319X-1M
831319X-1T
831319Y-1M
831319Y-1T
831320-4M
831320-4T
831321-1M
831321-1T
(a)
(a)
(a)
(a)

Figure 4.24. An example diagrammed to illustrate the procedures of the crosses in a comparison of the frequency of En61138-3-ILE derived from a pair of exceptional comparisons of main stalk and tiller (82 1143-6M vs. 1143-6T). Also see Table 4.51, lines 27-28, 39-40, 53-54, and 57-58. All females are a-m-1 sh/a-m-1 sh tester and all males are a-m En Sh/a-m-1 sh

1=Cross 4.4.2A
2=Cross 4.4.2B
3=Cross 4.4.2E
(+)=Kernels with loss sectors were selected
(-)=Kernels without loss sectors were selected
(a)=a pair of comparisons

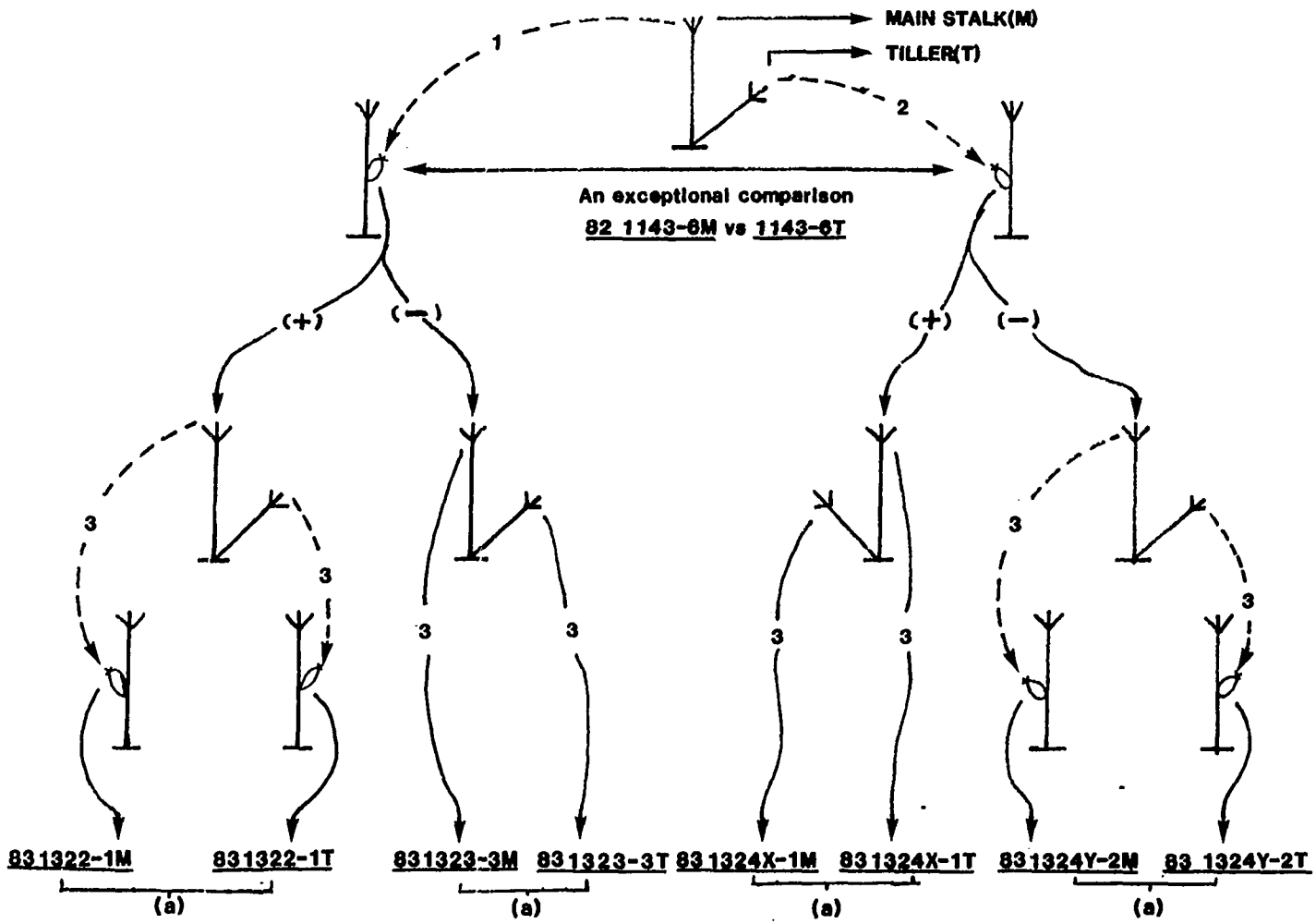

MAIN STALK(M)
TILLER(T)
1
2
An exceptional comparison
82 1143-6M vs 1143-6T
(+)
(−)
(+)
(−)
3
3
3
3
3
3
3
3
831322-1M
831322-1T
831323-3M
831323-3T
831324X-1M
831324X-1T
831324Y-2M
831324Y-2T
(a)
(a)
(a)
(a)

Figure 4.25. An example diagrammed to illustrate the procedures of the crosses in a comparison of the frequency of En61138-3-ILE derived from a pair of exceptional comparisons of main stalk and tiller (82 1144-5M vs. 1144-5T). Also see Table 4.51, lines 65-66, 75-76, 77-78, and 81-82. All females are a-m-1 sh/a-m-1 sh and all males are a-m En Sh/a-m-1 sh.

1=Cross 4.4.2A
2=Cross 4.4.2B
3=Cross 4.4.2E
(+)=Kernels with loss sectors were selected
(-)=Kernels without loss sectors were selected
(a)=a pair of comparisons

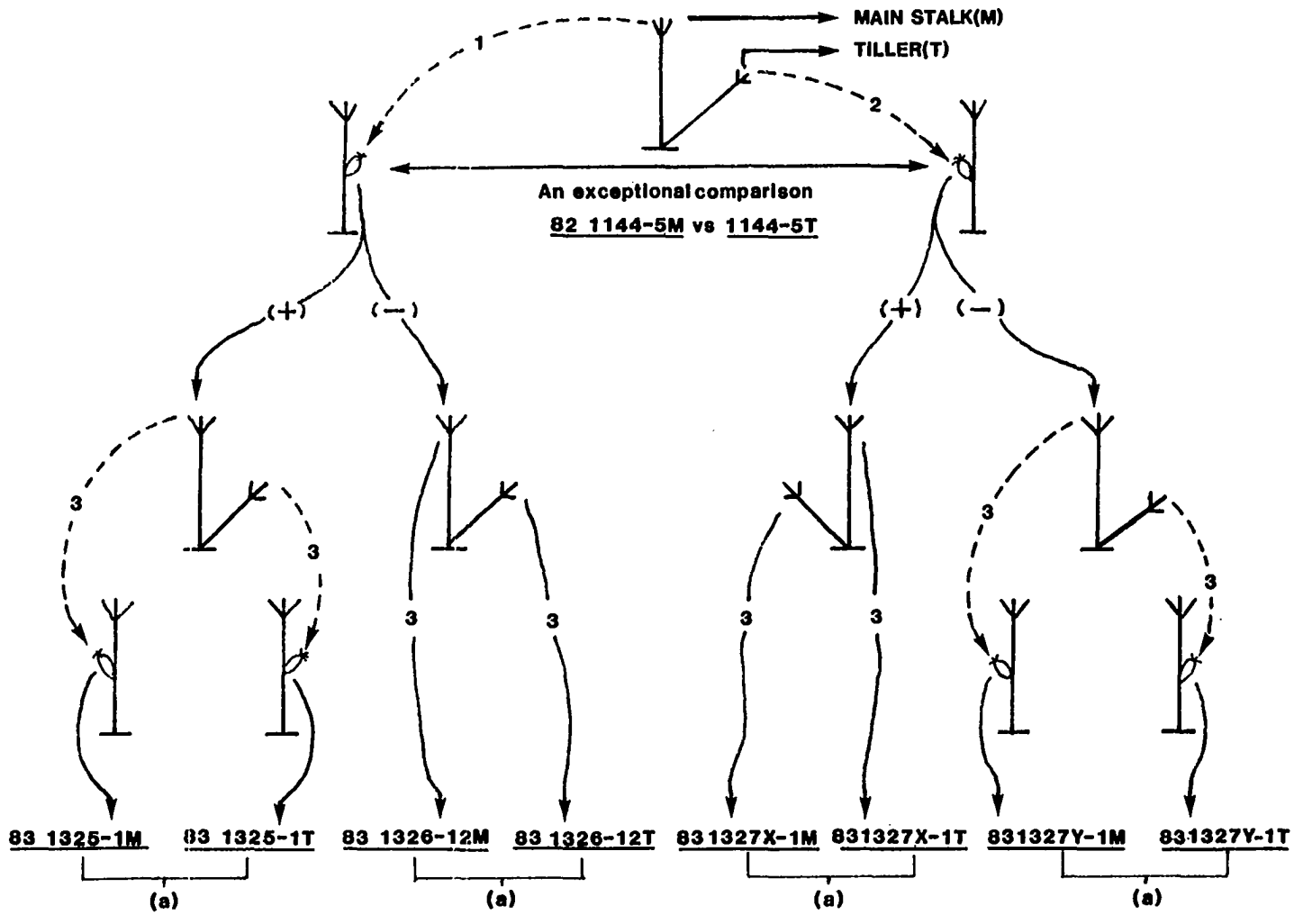

MAIN STALK(M)
TILLER(T)
1
2
An exceptional comparison
82 1144-5M vs 1144-5T
(+)
(−)
(+)
(−)
3
3
3
3
3
3
3
3
83 1325-1M
83 1325-1T
83 1326-12M
83 1326-12T
831327X-1M
831327X-1T
831327Y-1M
831327Y-1T
(a)
(a)
(a)
(a)

Table 4.51. Comparisons of the frequency of En61138-3 of the main stalk and tiller of the same plant in the testcross progeny derived from three exceptional comparisons (82 1142-5M vs. 1142-5T, 82 1143-6M vs. 1143-6T, and 82 1144-5M vs. 1144-5T)

Crosses[b] (progeny ear)	Round spotted[a] No loss	Round spotted[a] Loss	% of loss sectors	χ^2-value
A2. Parent 82 1142-5M (Table 4.9, line 9)				
1. 1319x-1M	49	1	1	0.0235
2. -1T	111	3	3	
3. y-1M	146	2	1	0.3379
4. -1T	123	0	0	
5. -4M	129	2	2	2.2923
6. -4T	191	11	5	
7. -5M	230	2	1	0.2157
8. -5T	162	0	0	
A2. Parent 82 1142-5T (Table 4.49, line 10)				
9. 1320-4M	108	1	1	0.0002
10. -4T	106	0	0	
11. -6M	157	3	2	0.1016
12. -6T	52	0	0	
13. 1321-1M	92	0	0	0.2154
14. -1T	130	2	2	
15. -4M	144	1	1	1.1928
16. -4T	22	0	0	
17. -6M	124	1	1	0.0017
18. -6T	38	1	3	
19. -7M	158	0	0	∞
20. -7T	127	0	0	

[a]The shrunken nonspotted colored kernels are not listed, but appear approximately ½ in all testcross progeny.

[b]1: 83 1319x, y seed source is from 82 1249/1142-5M with and without loss sectors respectively (Table 4.49, line 9); 83 1320, 1321 seed source is from 82 1312/1142-5T with and without loss sectors respectively (Table 4.49, line 10). 2: 83 1322, 1323 seed source is from 82 1249/1143-6M with and without loss sectors respectively (Table 4.49, line 21); 83 1324x, y seed source is from 82 1306/1143-6T with and without loss sectors respectively (Table 4.49, line 22). 3: 83 1325, 1326 seed source is from 82 1321/1144-5M with and without loss sectors respectively (Table 4.49, line 27); 83 1327x, y seed source is from 82 1315/1144-5T with and without loss sectors respectively (Table 4.49, line 28).

Table 4.51. Continued

Crosses (progeny ear)	Round spotted: No loss	Round spotted: Loss	% of loss sectors	χ^2-value
21. 1321-8M	143	1	1	0.0219
22. -8T	60	1	2	
23. -10M	135	0	0	0.0454
24. -10T	205	1	.1	
25. -11M	124	4	3	2.2384
26. -11T	126	0	0	
B1. Parent 82 1143-6M (Table 4.49, line 21)				
27. 1322-1M	195	0	0	16.9971**
28. -1T	30	4	12	
29. -2M	89	1	1	6.0729*
30. -2T	130	15	10	
31. -3M	89	0	0	4.2182*
32. -3T	186	12	6	
33. -5M	193	3	2	9.9249**
34. -5T	120	13	4	
35. -7M	137	0	0	4.6776*
36. -7T	232	11	4.5	
37. -10M	105	1	1	3.1225
38. -10T	173	11	6	
39. 1323-3M	85	0	0	1.8368
40. -3T	157	6	4	
41. -6M	56	2	3	0.0948
42. -6T	179	3	2	
43. -7M	143	13	8	1.2591
44. -7T	63	2	3	
45. -8M	200	3	1	0.0015
46. -8T	116	1	1	
47. -9M	83	0	0	0.0644
48. -9T	136	1	1	
49. -10M	40	0	0	0.7317
50. -10T	155	7	4	
51. -11M	61	4	6	0.5989
52. -11T	26	4	13	
B2. Parent 82 1143-6T (Table 4.49, line 22)				
53. 1324x-1M	36	0	0	1.0265
54. -1T	133	8	6	
55. -3M	148	0	0	0.2562
56. -3T	197	2	1	
57. y-2M	190	0	0	3.1587
58. -2T	189	5	3	

*,** Significant at .05 and .01, respectively.

Table 4.51. Continued

Crosses (progeny ear)	Round spotted: No loss	Round spotted: Loss	% of loss sectors	χ^2-value
59. 1324y-4M	174	2	1	0.9090
60. -4T	148	5	3	
61. -5M	112	1	1	0.1155
62. -5T	220	0	0	
63. -6M	145	2	1	5.6431*
64. -6T	170	14	8	
C1. Parent 82 1144-5M (Table 4.49, line 27)				
65. 1325-1M	155	2	1	0.0027
66. -1T	28	1	3	
67. -3M	66	0	0	0.5286
68. -3T	159	4	2	
69. -4M	93	0	0	∞
70. -4T	86	0	0	
71. -7M	130	0	0	∞
72. -7T	60	0	0	
73. -8M	136	3	2	0.0003
74. -8T	135	2	1	
75. 1326-12M	124	0	0	3.5602
76. -12T	192	8	4	
C2. Parent 82 1144-5T (Table 4.49, line 28)				
77. 1327x-1M	109	3	3	0.0394
78. -1T	92	2	2	
79 -2M	148	1	1	0.2784
80. -2T	193	1	1	
81. y-1M	134	3	2	0.0158
82. -1T	170	3	2	

the frequency of loss events. This variation results in an inconsistent expression of the loss events between main stalk and tiller.

From these results in Tables 4.51 and 4.52, it appears that the difference on the level of the loss event in the three exceptional comparisons is not heritable. The level of the loss event in the main

Table 4.52. Analysis of variance on the level of En61138-3 in the testcross progeny of the three exceptional derivations crossed onto a-m-1 sh/a-m-1 sh tester (Cross 4.4.2E)

	Source of variation	A[a]		B[a]		C[a]	
		df	MS	df	MS	df	MS
1	Stem[b]	1	.0089[ns]	1	.020[ns]	1	.0089*
2	Sectors (stem)[c]	2	.0014[ns]	2	.0032[ns]	2	.0004[ns]
3	Progeny stem[d] (sector/stem)	4	.0049[ns]	4	.0537[ns]	3	.0001[ns]
4	Error	18	.0058	30	.0080	11	.0069

[a]The seed source of Families A, B, and C is from main stalk ear and tiller ear of the same plant of the testcross progeny of 82 1142-5M, 1142-5T(A), 1143-6M, 1143-6T(B), 1144-5M, 1144-5T(C), respectively. See footnote of Table 4.51.

[b]Main stalk vs. tiller of the parental plant of Cross 4.4.2E.

[c]Kernels with colored shrunken sector vs. kernels without colored shrunken sector.

[d]Main stalk vs. tiller of the progeny of Cross 4.4.2E.

stalk and tiller is the same. The inconsistency of the results of 1982 and 1983 for these three exceptional comparisons are probably due to environmental effects and the content of the modifiers in the parental main stalk or tiller.

4.4.2.2.3. Effect of main stalk and tiller of the same plant derived from the tiller ear on En61138-3-ILE in 1983 Round spotted kernels with colorless background with and without sectors were selected and planted in 1983 from five parental tiller ears expressing a loss frequency from 19.4% to 0.91%.

Male gametes from the main stalk and tiller of these plants were pollinated onto the a-m-1 sh/a-m-1 sh tester plants (Cross 4.4.2F). The procedures for these crosses are similar with Crosses 4.4.2A, 4.4.2B, and 4.4.2E (see Figures 4.23-4.25). The results obtained by contingency χ^2-test for comparing the effect of main stalk and tiller on the frequency of the loss event showed that only three out of 38 comparisons exhibited a significant difference between the main stalk and tiller of the same plant. Two of these three comparisons had higher loss frequency from tiller than that from main stalk, one of these three had a higher loss frequency from main stalk than that from the tiller (Table 4.53, lines 1-2, 9-10, 21-22).

Both of the results obtained in Table 4.49 and Table 4.53 showed that the male gametes containing the En61138-3 allele from parental main stalk or parental tiller are capable of expressing a similar level of En61138-3-ILE in their testcross progeny. No significant difference was noticed between the main stalk and tiller of the same plant.

Table 4.53. Contingency χ^2-test on the frequency of En61138-3 of main stalk and tiller in the testcross progeny of the parental tiller crossed onto a-m-1 sh/a-m-1 sh tester (Cross 4.4.2F)

Crosses[b] (1983 ♂ number)	Round spotted[a] No loss	Loss	% of loss sector	χ^2-value
1. 1309-1M	105	1	1	10.97**
2. -1T	109	17	18	
3. -2M	104	1	1	0.14
4. -2T	116	3	3	
5. -3M	37	1	3	0.06
6. -3T	198	7	3	
7. -6M	104	1	1	1.68
8. -6T	169	8	5	
9. -7M	157	4	2	6.76**
10. -7T	89	11	11	
11. 1310-3M	136	0	0	∞
12. -3T	49	0	0	
13. -4M	198	8	4	0.0181
14. -4T	96	5	5	
15. -5M	76	2	3	1.0442
16. -5T	237	1	0.4	
17. -6M	126	0	0	0.2503
18. -6T	169	2	1	
19. -8M	94	3	3	0.417
20. -8T	168	2	1	
21. -9M	69	5	7	5.7940*
22. -9T	119	0	0	

[a]The shrunken nonspotted colored kernels are not listed but appear approximately ½ in all the progeny.

[b]83 1309, 1310 seed source is from the ear 82 1302/111-7T with and without loss sectors respectively (data is not shown). 83 1311, 1312 seed source is from the ear 82 1312/1141-1t with and without loss sectors respectively (Table 4.19, line 1). 83 1313, 1314 seed source is from the ear 82 1317/1155-9t with and without loss sectors respectively (Table 4.26, line 20). 83 1315, 1316 seed source is from the ear 82 1315/1147-1t with and without loss sectors respectively (Table 4.27, line 2). 83 1317, 1318 seed source is from the bulk seed of a-m-1 sh/a-m-1 sh tester crossed by 82 1142-7t, 1146-4t, 1147-8t, 1150-3t, 1155-4t, 1155-7t, 1156-5t, 1157-1t, 1157-3t (Table 4.19, line 25; Table 4.21, line 19; Table 4.22, line 6; Table 4.23, line 8; Table 4.26, lines 10, 19, 26; Table 4.27, lines 2, 7).

**,*Significant at .01 and .05, respectively.

Table 4.53. Continued

Crosses (1983 ♂ number)	Round spotted: No loss	Round spotted: Loss	% of loss sector	χ^2-value
23. 1310-10M	33	0	0	0.1834
24. -10T	194	2	1	
25. -11M	161	0	0	0.1834
26. -11T	140	3	2	
27. -12M	157	0	0	3.3297
28. -12T	113	4	3	
29. 1311-7M	107	0	0	2.5414
30. -7T	186	7	4	
31. -8M	119	5	4	0.2158
32. -8T	164	4	2	
33. 1312-5M	60	0	0	2.1717
34. -5T	97	6	6	
35. -6M	153	0	0	1.9692
36. -6T	63	2	3	
37. -10M	81	0	0	0.2023
38. -10T	33	1	3	
39. 1313-1M	124	4	3	0.1952
40. -1T	129	2	2	
41. -6M	180	2	1	0.1249
42. -6T	107	0	0	
43. 1314-2M	110	0	0	0
44. -2T	109	1	1	
45. -5M	100	2	2	0.4782
46. -5T	53	3	5	
47. -6M	99	0	0	0.0101
48. -6T	421	3	0.7	
49. -9M	159	3	2	0.3242
50. -9T	172	1	1	
51. 1315-2M	140	1	1	0.1831
52. -2T	92	1	1	
53. -5M	82	0	0	0.3133
54. -5T	100	2	2	
55. -6M	119	2	2	0.0997
56. -6T	67	0	0	
57. 1316-2M	123	0	0	∞
58. -2T	69	0	0	

Table 4.53. Continued

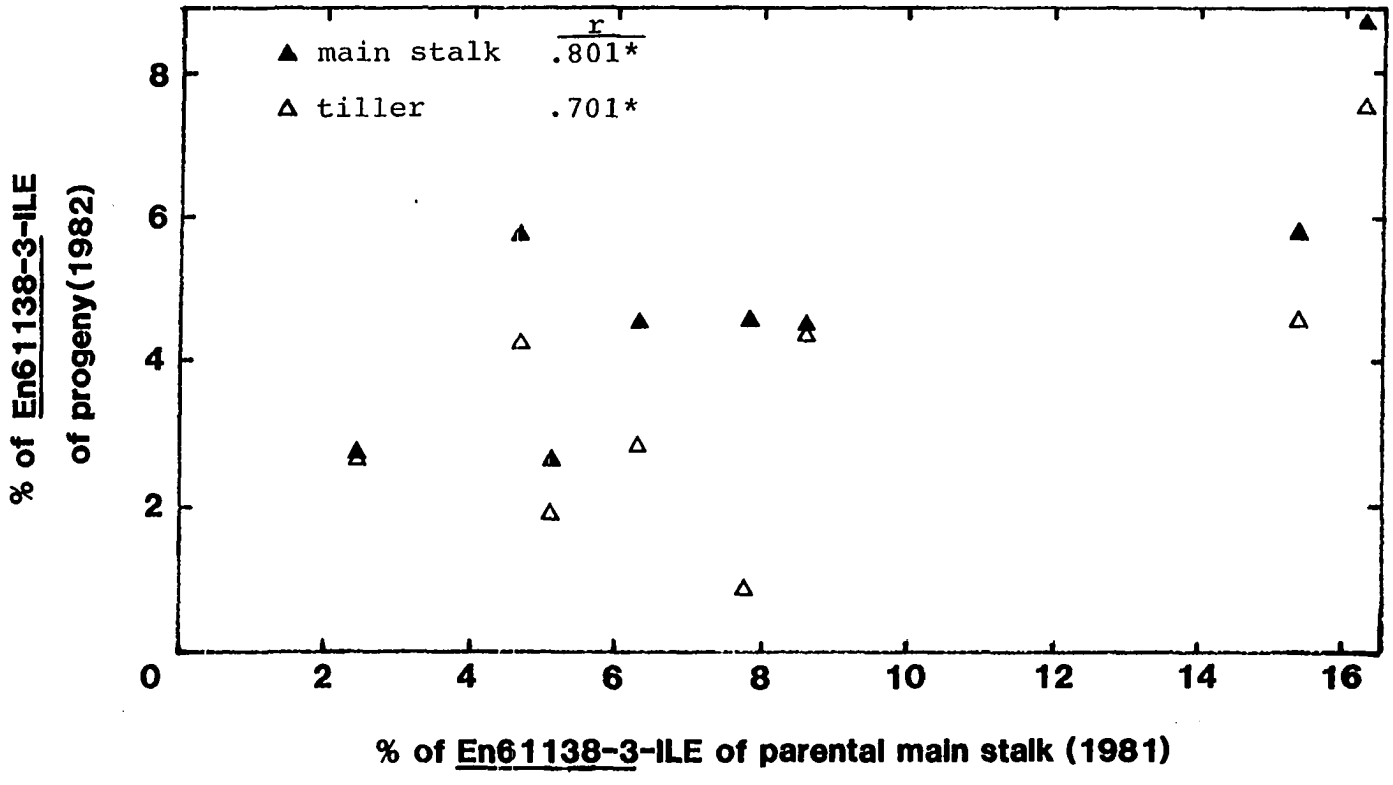
Crosses (1982 ♂ number)	Round spotted No loss	Round spotted Loss	% of loss sector	χ^2-value
59. 1316-10M	94	1	1	0
60. -10T	94	1	1	
61. -11M	189	1	1	0.6641
62. -11T	43	0	0	
63. 1317-4M	140	0	0	∞
64. -4T	127	0	0	
65. -5M	98	2	2	0.3644
66. -5T	305	2	0.7	
67. -7M	107	1	1	0.6872
68. -7T	76	3	4	
69. -8M	51	1	2	0.0116
70. -8T	42	0	0	
71. 1318-6M	54	1	2	0.0185
72. -6T	42	0	0	
73. -7M	61	0	0	0.2698
74. -7T	164	1	1	
75. -8M	151	1	1	0.4838
76. -8T	154	1	1	

4.4.2.3. Heritability of the En61138-3 phenotype through main stalk and tiller of the same plant

4.4.2.3.1. Parent-offspring correlation

4.4.2.3.1.1. Heritability of En61138-3 phenotype through parental main stalk The results from the testcross progeny of eight families in 1982 (Cross 4.2.1B) showed that there was a significant positive correlation (r=.8014*) between the frequency of En61138-3-ILE of the parental main stalk and that of their corresponding progeny main stalk (Figure 4.26).

Figure 4.26. Correlation of the frequency of En61138-3-ILE between the parental main stalk and that of their corresponding progeny main stalk and tiller


r
▲ main stalk .801*
△ tiller .701*
% of En61138-3-ILE of progeny(1982)
% of En61138-3-ILE of parental main stalk (1981)
0
2
4
6
8
10
12
14
16

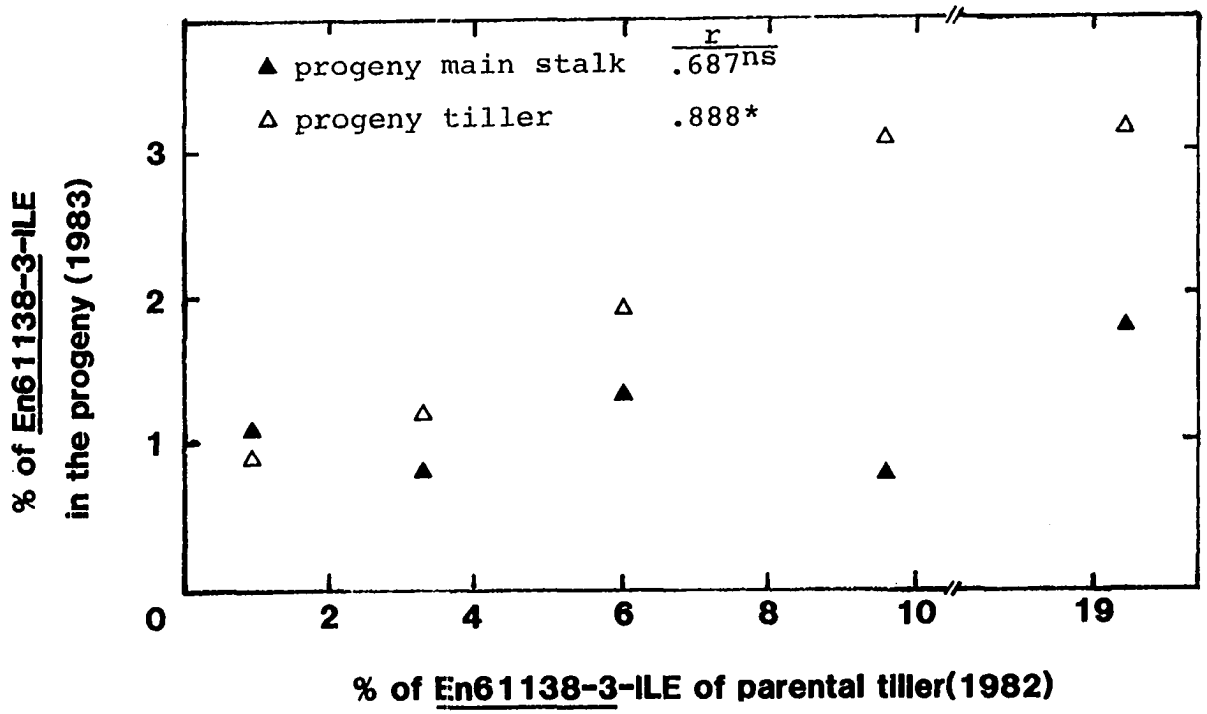
A significant positive correlation (r=.7016*) was also found between the frequency of En61138-3-ILE of the parental main stalk and that of their corresponding progeny tiller (Figure 4.26).

This relationship indicates that the level of En61138-3-ILE can be transmitted to the progeny main stalk (80.14% heritability) and progeny tiller (70.16% heritability) through their corresponding parental main stalk.

4.4.2.3.1.2. Heritability of En61138-3-ILE through parental tiller The heritability of En61138-3-ILE through the parental tiller to their progeny main stalk and tiller could be analyzed from the same testcross progeny of the five families in 1983 (Cross 4.4.2F, section 4.4.2.2.3., Table 4.53). The results are shown in Figure 4.27. It was found that a significant positive correlation (r=.8876*) exists between the frequency of En61138-3-ILE of parental tiller and that of their corresponding progeny tiller. This suggested that the relative level of En61138-3-ILE can be transmitted to the progeny tiller through their parental tiller. However, the relative level of En61138-3-ILE did not appear as strongly in the progeny main stalk at the same level as that in the progeny tiller. Here, the heritability was 68.73% vs. 88.76% in the progeny tiller (Figure 4.27).

4.4.2.3.2. Contingency χ^2-test In an attempt to assess the heritability of En61138-3 on an individual plant basis within each family through parental main stalk or tiller to their progeny main stalk or tiller, the same data of the five families of 1983 (Table 4.53) with their corresponding parental tillers were analyzed by contingency χ^2-test.

Figure 4.27. Correlation of the frequency of En61138-3 between the parental tiller and their corresponding progeny main stalk and tiller


r
▲ progeny main stalk .687ns
△ progeny tiller .888*
% of En61138-3-ILE in the progeny (1983)
0
1
2
3
2
4
6
8
10
19
% of En61138-3-ILE of parental tiller(1982)

The results are shown in Table 4.54. The percentage of nonsignificant χ^2 was estimated to represent the heritability of En61138-3-ILE from parental tillers to their progeny as follows: Higher percentage of nonsignificant χ^2-values shows higher heritability.

$$\% \text{ of nonsignificant } \chi^2 = \frac{\text{no. of the comparisons with nonsignificant } \chi^2 \text{ in one family}}{\text{total no. of the comparisons in one family}} \times 100$$

The results of Table 4.54 showed that the frequency of En61138-3-ILE was less than 6% in parental tiller ears then the 50% to 100% of nonsignificant χ^2 were found in the comparisons of the progeny main stalk with their parental tillers (Table 4.54, columns 3, 4, 5). However, the percentages of nonsignificant χ^2 are only 20% in family 83 1311-1312(M) and 0% in family 83 1309-1310(M) in the comparisons of the progeny main stalk with their corresponding parental tiller which expresses a high frequency of En61138-3-ILE (>9%) (Table 4.54, columns 1, 2).

In a comparison of the frequency of the loss events in the parental tillers with their corresponding progeny tillers, it is evident that a similar trend can be seen as presented with the parental tiller vs. progeny main stalk comparison (Table 4.54). However, 80% of the tillers in family 83 1311-1312(T) showed the same level of the loss events as their parental tillers which exhibited a high frequency of the loss event (9.57%) and only 20% of the nonsignificant χ^2 values were found in the progeny main stalk vs. parental tiller comparisons within this family (Table 4.54, column 2).

From these results, it indicated that the frequency of En61138-3-ILE could be more strongly transmitted to the progeny main stalk or tiller

Table 4.54. Percentage of nonsignificant χ^2-values in the comparisons of the parental tillers and their corresponding progeny main stalks and tillers by contingency χ^2-test

	Parental tiller ear[a] (1982)	(1) 1111-1T (19.4%)[b]	(2) 1141-1T (9.57%)	(3) 1155-9T (5.97%)	(4) 1147-1T (3.29%)	(5) bulked seed (0.91%)
	Progeny[a] (1983)	1309-1310	1311-1312	1313-1314	1315-1316	1317-1318
Progeny	Main stalk (M)	0	20	50	100	100
	Tiller (T)	14.3	80	50	100	100

[a]The source of parents and progeny are described in the footnote of Table 4.53.

[b]Numbers in the parentheses is the frequency of En61138-3-ILE.

through their parental tillers with lower frequency of the loss events than through the parents with a high frequency of the loss events.

Four out of the five families showed 50% or more nonsignificant χ^2-values in the parental tiller vs. progeny tiller comparisons (Table 4.54, columns 2, 3, 4, 5) and only three out of the five families showed 50% or more nonsignificant χ^2-values in parental tiller vs. progeny main stalk (Table 4.54, columns 3, 4, 5). This is probably the basis for the higher correlation between the frequency of En61138-3-ILE of the parental tillers and that of the progeny tillers compared with the frequency of En61138-3-ILE between parental tillers and their progeny main stalks on family basis ($r=.8876^{*}$ vs. $r=.6873^{ns}$, Figure 4.27).

Another set of data was used to measure the heritability of the En61138-3-ILE from parental main stalk or tiller to progeny main stalk or tiller by contingency χ^2-test. This set of data is the same as that of Table 4.51. The results of contingency χ^2-test are presented in Table 4.55. It was found that a higher frequency of loss events in the parental main stalk or tiller is correlated with a lower percentage of nonsignificant χ^2-values in the progeny main stalk or tiller (Table 4.55, columns 2, 3, 5). This means that the parental main stalk or tillers expressing a low frequency of the loss event could transmit their potential for the same or approximately equal level of the loss event to their corresponding progeny main stalks and tillers (Table 4.55, columns 1, 4, 6). These results are consistent with that of Table 4.54. The higher heritability of the frequency of En61138-3-ILE can be expressed within each family through the parent with a lower frequency of En61138-3-ILE to its individual progeny plants.

Table 4.55. Percentages of nonsignificant χ^2-values in the comparisons of the parental main stalks and tillers and their corresponding progeny main stalks and tillers by contingency χ^2-test

Parent[a] (1982)	1142-5		1143-6		1144-5	
	M(.7%)[b] (1)	T(6.0%) (2)	M(16.1%) (3)	T(3.1%) (4)	M(11.1%) (5)	T(2.1%) (6)
Progeny[a] (1983)	1319x-1319y	1320-1321	1322-1323	1324x-1324y	1325-1326	1327x-1327y
M[c]	100	30.8	15.4	83.3	23.1	100
T[c]	75	55.6	30.8	83.3	33.3	100

[a]The source of parents and progeny are shown in the footnote of Table 4.51.

[b]Numbers in the parentheses is the frequency of En61138-3-ILE.

[c]M: Main stalk; T: Tiller.

The inconsistency of the heritability of the En61138-3-ILE between the progeny main stalk and tiller derived from the parental main stalk or tiller in three families (family 83 1320-1321, 83 1322-1323, 83 1325-1326, Table 4.55, columns 2, 3, 5) might be dependent on the segregation of modifiers within these progeny plants. In addition, this segregation of modifiers could be implied to explain the low heritability of the frequency of En61138-3-ILE in these three families (Table 4.55, columns 2, 3, 5) and the two families (83 1309-1310 and 83 1311-1312 in Table 4.54, columns 1, 2).

4.4.2.4. Comparison of the states of En61138-3-ILE in main stalk and tiller The results of the selection of states of En61138-3-ILE in the testcross progeny of the main stalk and tiller in 1982 are presented in Table 4.56. It was found that the frequencies of each of the sizes of the loss events in main stalk were higher than that in tiller, but the difference was not significant by t-test. This indicates that the frequency of the occurrence of the loss events at a specific time in main stalk and tiller was the same.

Similar results were obtained in 1983, though the frequency of different sizes of the loss sectors in the tiller was slightly higher than that in the main stalk, yet no significant difference on the states of En61138-3-ILE between main stalk and tiller was found (Table 4.56).

4.4.3. Planting date effects on En61138-3-ILE

It has been reported that environmental factors such as temperature could effectively influence the rate of mutation in the mutable systems of maize (Rhoades, 1941; Peterson, 1958). Significant environmental

Table 4.56. Comparisons of the states of En61138-3 in main stalk and tiller by t-test

Year	No. of comparisons	States of En61138-3					
		Small		Medium		Large	
		Main	Tiller	Main	Tiller	Main	Tiller
1982	14	6.79±1.12	5.49±.99	.29±.15	0	.27±.13	.04±.04
	t-value =	.8758[ns][a]		1.9986[ns]		1.5536[ns]	
1983	38	1.16±.25	1.83±.44	.01±.01	.06±.04	.14±.07	.25±.10
	t-value =	-1.3204[ns]		-1.1966[ns]		-.8197[ns]	

[a]ns = Nonsignificant.

influences, such as planting date, have been reported on grain yield (corn, oats, soybean, safflower, sunflower, etc.), seed quality, and oil content (soybean, sunflower) (Genter and Jones, 1970; Schmidt, 1960; Green et al., 1965; Luebs et al., 1965; Unger, 1980). Other examples include the increase of meiotic irregularities in five advanced hexaploid triticales with delayed planting was found by Sisodia et al. (1970). However, there is no report on the effect of planting date on the mutation rate in maize mutable systems. Therefore, the purpose of this experiment is to investigate the effects of planting dates on the frequency of En61138-3-ILE.

Round spotted kernels with colorless background with and without loss sectors were selected from two parental ears (81 0911/1032-8t and 81 0921/1032-8t) and a bulked sample from ears with low frequency of loss sectors (Table 4.13, lines 19, 18 and Table 4.13, lines 12, 31; Table 4.14, lines 4, 6, 21, 33, 50, 51; Table 4.16, lines 4, 6, 15, 18, 19; Table 4.17, lines 7, 24; Table 4.18, lines 2, 5, 10, 14, 15). These three parental samples containing the genotype a-m En Sh/a-m-1 sh (En61138-3) have their own frequencies of loss events; namely 38.2%, 12.8%, and 1.04% (weighted value). Plants derived from these kernels were grown at two different planting dates (May 7 and May 24, 1982) at the Brunnier Agronomy Farm near Ames and were assayed for loss events by crossing onto the a-m-1 sh/a-m-1 sh tester. The frequencies of En61138-3-ILE among these testcross progeny are shown in Table 4.57. The results of analysis of variance showed that there was not significant difference between these two planting dates on the frequency of

Table 4.57. Effect of planting dates on the frequency of En61138-3-ILE in the progeny of a-m-1 sh/ a-m-1 sh x a-m En Sh/a-m-1 sh

Planting Date[b] \ Family[a]	1			2			3		
	+[c]	-c	X±SE	+	-	X±SE	+	-	X±SE
1	9.21±5.04	4.75±1.59	6.47±2.14	17.88±2.61	2.86±1.09	10.37±2.64	2.87	2.24±0.52	2.55±1.31
2	8.91±2.60	4.21±2.60	7.34	12.11±4.80	2.09±0.96	7.66±3.10	3.73±0.95	1.26±0.50	2.58±0.63

[a]Seed sources: Family 1 was derived from ear 81 0911/1032-8t (Table 4.13, line 19); Family 2 was derived from ear 81 0921/1032-8t (Table 4.13, line 18); Family 3 was derived from a bulked sample of the kernels with low frequency of En61138-3-ILE. (Table 4.13, lines 12, 31; Table 4.14, lines 4, 6, 21, 33, 50, 51; Table 4.16, lines 4, 6, 15, 18, 19; Table 4.17, lines 7, 24; Table 4.18, lines 2, 5, 10, 14, 15).

[b]Planting date 1: May 8, 1982; Planting date 2: May 24, 1982.

[c]+ represents kernels with loss sectors; - represents kernels without loss sectors.

En61138-3-ILE (Table 4.58). But the difference between the kernels with and without loss sectors on the frequency of En61138-3-ILE is highly significant. Thus, the effect of 15-day difference of the planting date has no significant effect on the level of En61138-3-ILE.

4.5. Response to Selection on En61138-3

4.5.1. Response to selection on En61138-3-ILE in three successive years

Through three successive years of selection (1981 to 1983) for manipulating the frequency of En61138-3-ILE, it was evident that the mean of the frequency of En61138-3-ILE decreased rapidly from 1981 to 1983. The result is shown in Figure 4.28. The main reason is the dilution of the frequencies of the modifiers in the testcross progeny (see section 4.4.1.4). It is assumed that the modifiers are not included in the female parents (a-m-1 sh/a-m-1 sh) of the testcross progeny. The frequency of En61138-3-ILE might be directed toward its base line as the testcross continues. The rapid decrease of the frequency of the loss event from 1982 to 1983 is probably due to the environmental effects and the selection directions in addition to the dilution of the modifiers. Moreover, the bidirectional selection executing in 1982 might also result in lower frequency of En61138-3-ILE in 1983 compared with that in 1982. The selection scheme will be given in the following section.

4.5.2. Bidirectional selection on En61138-3-ILE

4.5.2.1. Response to bidirectional selection on En61138-3-ILE

A bidirectional plan is shown in Figure 4.29 to Figure 4.33.

Individual plants with high and low frequencies of loss events in each family were selected and crossed onto a-m-1 sh/a-m-1 sh tester to

Figure 4.28. Changes of the frequency of En61138-3-ILE in three successive selections (1981 to 1983)

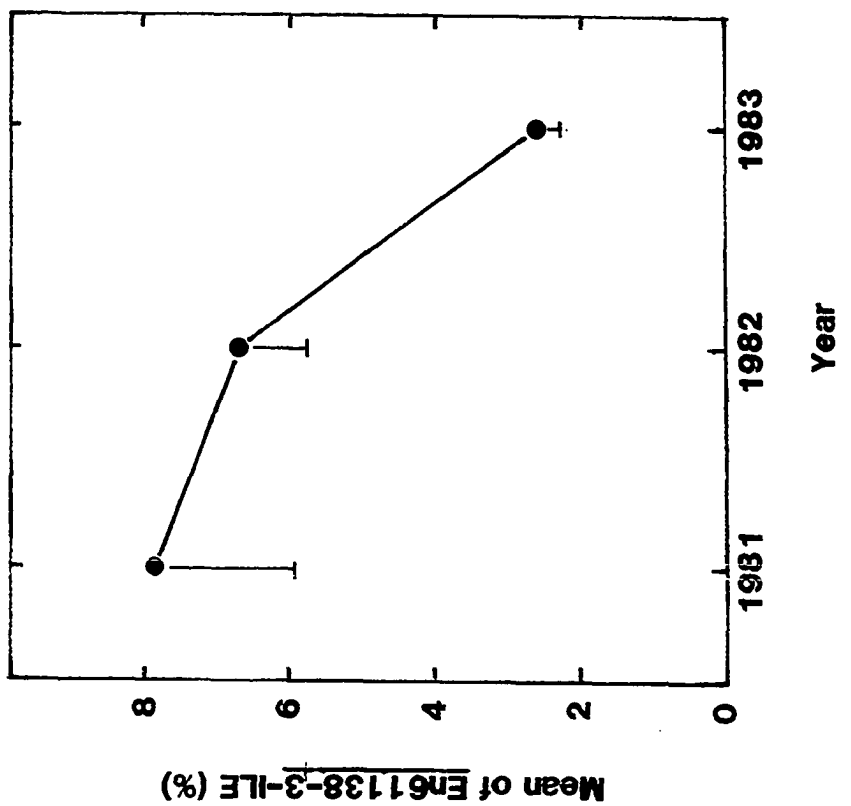

Mean of En6138-3-ILE (%)
0
2
4
6
8
1931
1982
1983
Year

Figure 4.29. Bidirectional selection scheme on the frequency of En61138-3-ILE. All the progeny were derived from the crosses of the plants containing the En61138-3 allele and the a-m-1 sh/a-m-1 sh tester (Crosses 4.2.1A and 4.2.1B)

(+) round spotted kernels with loss sectors were selected
(-) round spotted kernels without loss sectors were selected

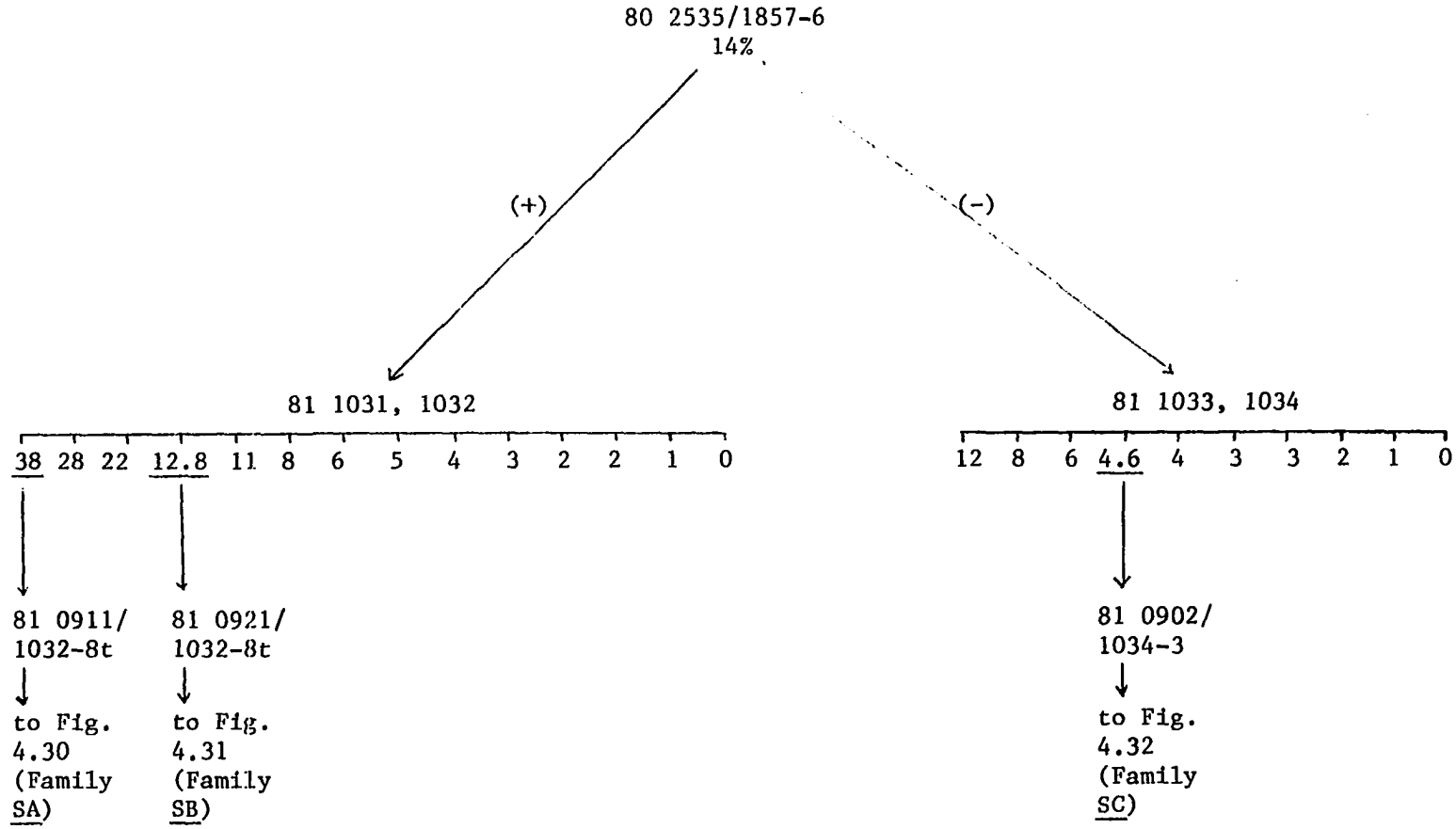

80 2535/1857-6
14%
(+)
(-)
81 1031, 1032
38 28 22 12.8 11 8 6 5 4 3 2 2 1 0
81 1033, 1034
12 8 6 4.6 4 3 3 2 1 0
81 0911/
1032-8t
to Fig.
4.30
(Family
SA)
81 0921/
1032-8t
to Fig.
4.31
(Family
SB)
81 0902/
1034-3
to Fig.
4.32
(Family
SC)

Figure 4.30. Response of bidirectional selection on the frequency of En61138-3-ILE in Family SA derived from 81 0911/1032-8t

(+) (-): see Figure 4.29

---

[a] Frequency of En61138-3-ILE of ear 81 0911/1032-8t (Table 4.13, line 19).

[b] SA-1 represents the 83 1241 family, SA-2 represents the 83 1242 family, and so on.

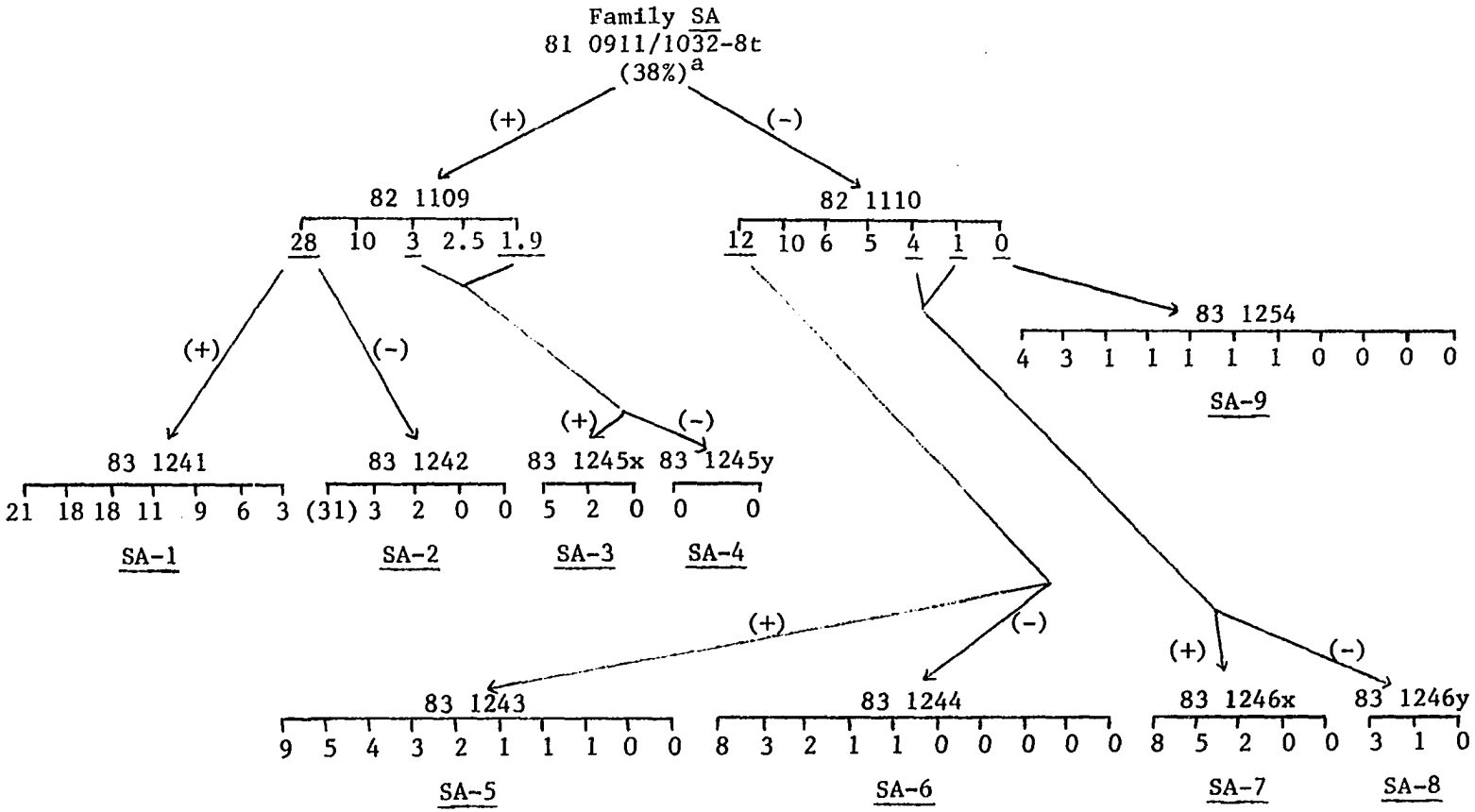

Family SA
81 0911/1032-8t
(38%)a
(+)
(-)
82 1109
28 10 3 2.5 1.9
82 1110
12 10 6 5 4 1 0
(+)
(-)
83 1254
4 3 1 1 1 1 1 0 0 0 0
SA-9
(+)
(-)
83 1241
21 18 18 11 9 6 3
SA-1
83 1242
(31) 3 2 0 0
SA-2
83 1245x
5 2 0
SA-3
83 1245y
0 0
SA-4
(+)
(-)
(+)
(-)
(+)
(-)
83 1243
9 5 4 3 2 1 1 1 0 0
SA-5
83 1244
8 3 2 1 1 0 0 0 0 0
SA-6
83 1246x
8 5 2 0 0
SA-7
83 1246y
3 1 0
SA-8

Figure 4.31. Response of selection on the frequency of En61138-3-ILE in Family SB derived from 81 0921/1032-8t

(+) (-): see Figure 4.27

---

[a]Frequency of En61138-3-ILE of ear 81 0921/1032-8t (Table 4.13, line 18).

[b]SB-1 represents the 83 1247 family, SB-2 represents the 83 1248 family, and so on.

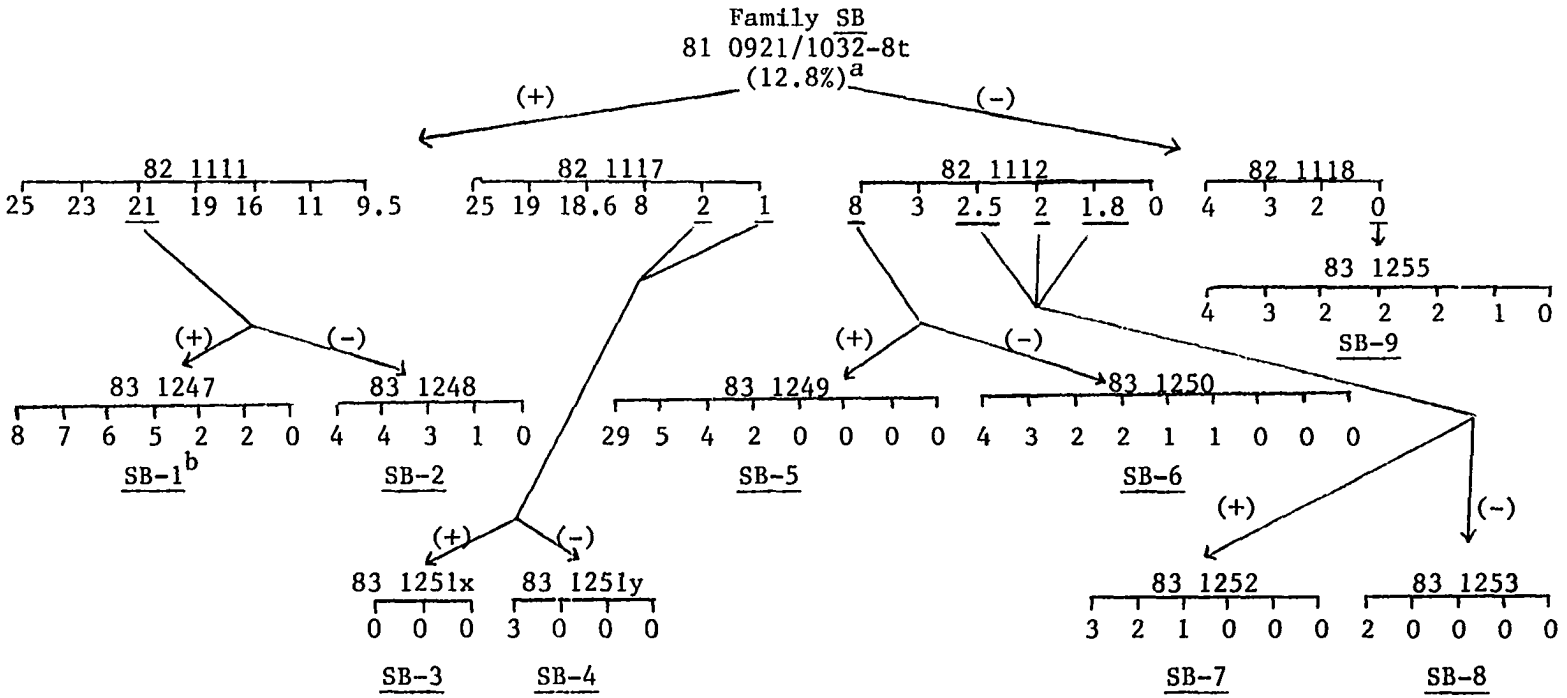

Family SB
81 0921/1032-8t
(12.8%)a
(+)
(-)
82 1111
25 23 21 19 16 11 9.5
82 1117
25 19 18.6 8 2 1
82 1112
8 3 2.5 2 1.8 0
82 1118
4 3 2 0
83 1255
4 3 2 2 2 1 0
SB-9
(+)
(-)
83 1247
8 7 6 5 2 2 0
SB-1b
83 1248
4 4 3 1 0
SB-2
83 1249
29 5 4 2 0 0 0 0
SB-5
83 1250
4 3 2 2 1 1 0 0 0
SB-6
(+)
(-)
83 1251x
0 0 0
SB-3
83 1251y
3 0 0 0
SB-4
(+)
(-)
83 1252
3 2 1 0 0 0
SB-7
83 1253
2 0 0 0 0
SB-8

Figure 4.32. Response of selection on the frequency of En61138-3-ILE in Family SC derived from 81 0902/1034-3

(+) (-): see Figure 4.29

---

[a] Frequency of En61138-3-ILE of ear 81 0902/1034-3 (Table 4.13, line 32).

[b] SC-1 represents the 83 1301 family, SC-2 represents the 83 1302 family, and so on.

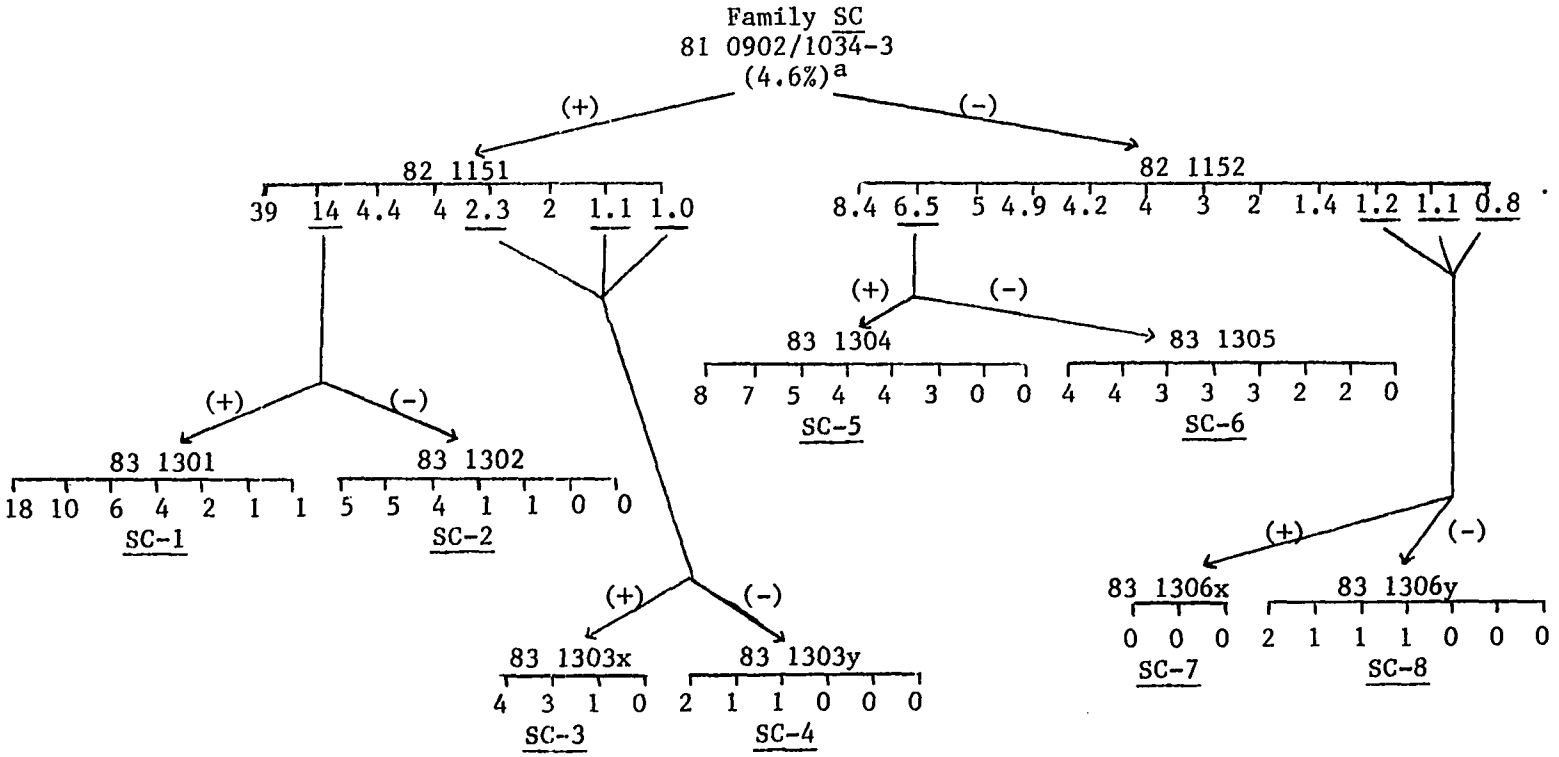

Family SC
81 0902/1034-3
(4.6%)a
(+)
(-)
82 1151
39 14 4.4 4 2.3 2 1.1 1.0
82 1152
8.4 6.5 5 4.9 4.2 4 3 2 1.4 1.2 1.1 0.8
(+)
(-)
83 1304
8 7 5 4 4 3 0 0
SC-5
83 1305
4 4 3 3 3 2 2 0
SC-6
(+)
(-)
83 1301
18 10 6 4 2 1 1
SC-1
83 1302
5 5 4 1 1 0 0
SC-2
(+)
(-)
83 1303x
4 3 1 0
SC-3
83 1303y
2 1 1 0 0 0
SC-4
(+)
(-)
83 1306x
0 0 0
SC-7
83 1306y
2 1 1 1 0 0 0
SC-8

Figure 4.33. Response of selection on the "zero" frequency of En61138-3-ILE in Family SD derived from a bulked sample [(+) and (-): see Figure 42.9]

---

[a]Frequency of En61138-3-ILE of the bulked sample (see footnote of Table 4.18).

[b]Frequency of En61138-3-ILE of the bulked sample (see footnote of Table 4.57, Family 3).

[c]SD-1 represents the 83 1307 family; SD-2 represents the 83 1308 family.

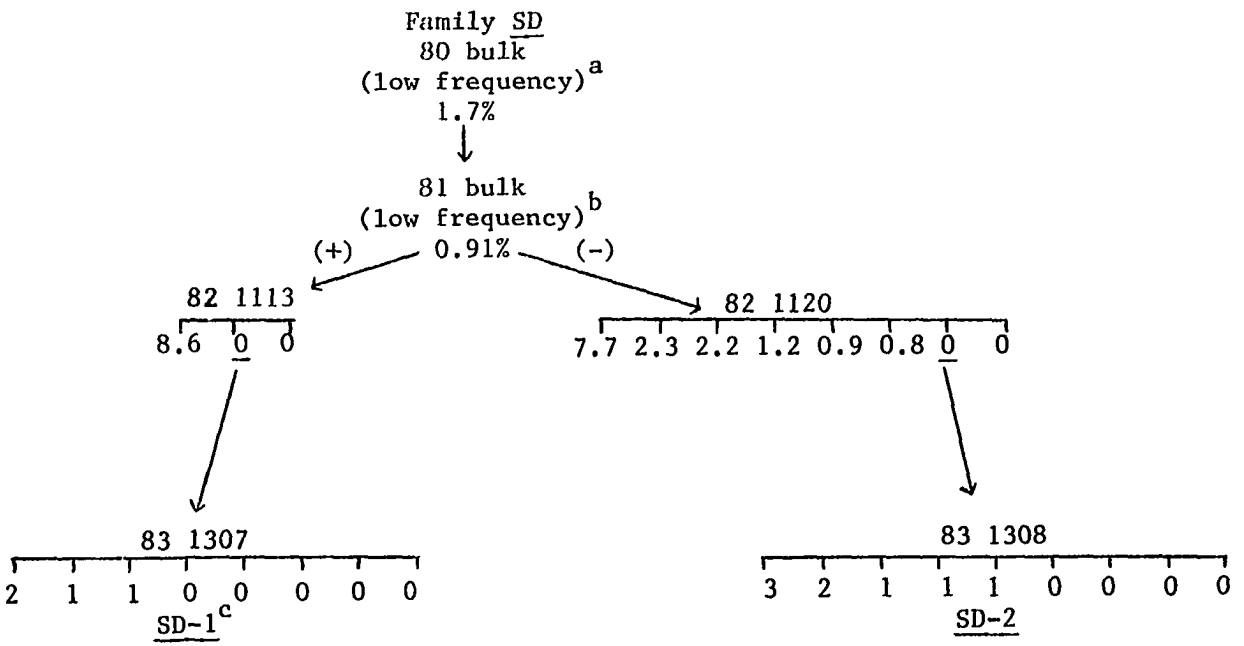

Family SD
80 bulk
(low frequency)a
1.7%
81 bulk
(low frequency)b
(+) 0.91% (-)
82 1113
8.6 0 0
82 1120
7.7 2.3 2.2 1.2 0.9 0.8 0 0
83 1307
2 1 1 0 0 0 0 0
SD-1c
83 1308
3 2 1 1 1 0 0 0 0
SD-2

Table 4.58. Analysis of variance for the effect of planting dates on the frequency of En61138-3-ILE in three families[a] derived from the crosses of a-m-1 sh/a-m-1 sh tester and a-m En Sh/a-m-1 sh (En61138-3)

Source of variation	df	MS
Planting dates	1	81.0960[ns b]
Families (planting dates)	4	412.0873[ns]
Sectors[c] (families/planting dates)	6	762.9897**
Error	1076	28.2229

[a]Source of these families are shown in footnote of Table 4.57.

[b]ns = Nonsignificant.

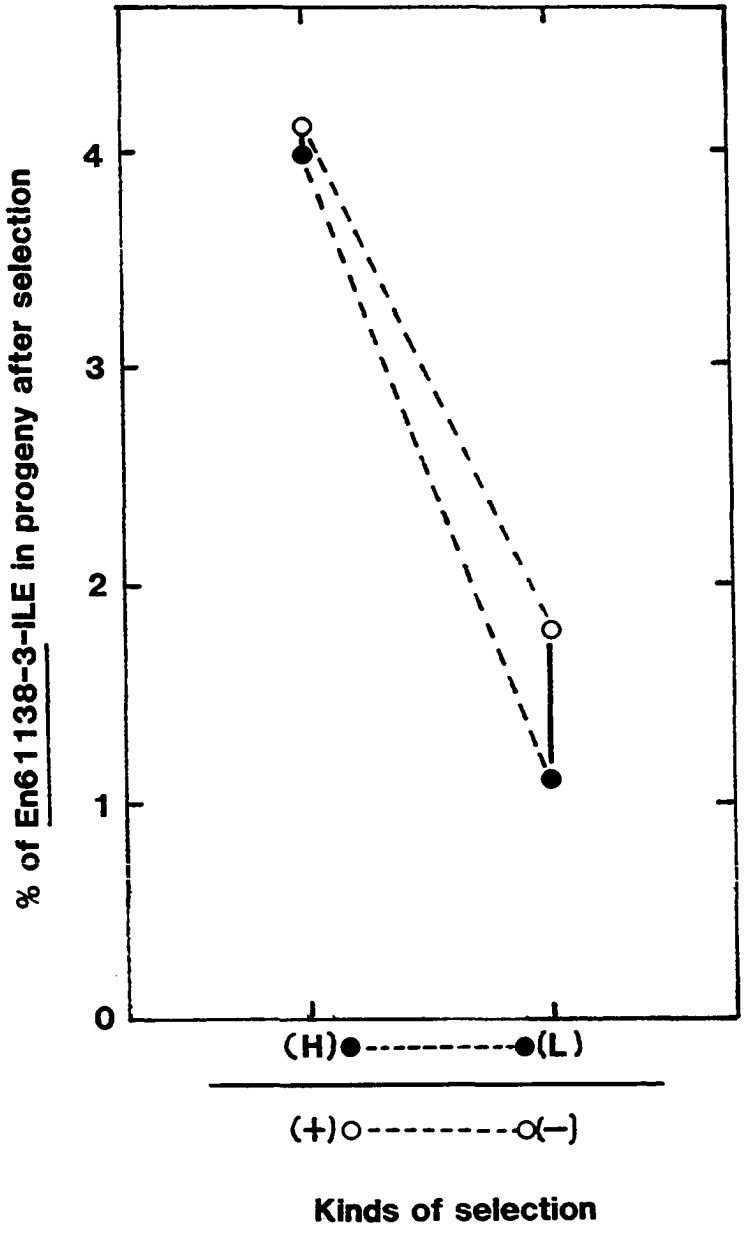
[c]Sectors represent kernels with colored shrunken sectors vs. kernels without colored shrunken sectors.

**Significant at .01 by F-test.

yield testcross progeny. These testcross progeny derived from parents with a high frequency of loss events (families SA-1, SA-2, SA-5, SA-6 of Figure 4.30; families SB-1, SB-2, SB-5, SB-6 of Figure 4.31; families SC-1, SC-2, SC-5, SC-6 of Figure 4.32) are classified in the (H) class of the loss events. Families of SA-3, SA-4, SA-7, SA-8 of Figure 4.30; SB-3, SB-4, SB-7, SB-8 of Figure 43.1; SC-3, SC-4, SC-7, SC-8 of Figure 4.32 derived from the parents with low frequency of En61138-3-ILE are classified in the (L) class of the loss events. The results of these selections shown in Figure 4.34 indicate that high (H) and low (L) loss frequencies of the loss event decreased in the testcross progeny compared with their parental testcross populations (see Figure 4.28).

Figure 4.34. Responses to bidirectional selection on En61138-3-ILE in two kinds of selection

——— Shows no significant difference by t-test
_____ Shows highly significant difference at 1% level by t-test


% of En61138-3-ILE in progeny after selection
4
3
2
1
0
(H)●----------●(L)
(+)○--------○(–)
Kinds of selection

The difference in the frequency of the loss event between the selection for (H) and for (L) was highly significant. The frequency of the loss event in (H) class is about four times higher than that in (L) class (Figure 4.34).

Individual plants derived from kernels with colored shrunken sectors (+) and without colored shrunken sectors (-) were selected respectively in each family. Families SA-1, SA-3, SA-5, SA-7, SB-1, SB-3, SB-5, SB-7, SC-1, SC-3, SC-5, SC-7 are (+) class. Families SA-2, SA-4, SA-6, SA-8, SB-2, SB-4, SB-6, SB-8, SC-2, SC-4, SC-6, and SC-8 are (-) class. A highly significant response of the frequency of the loss events to selection on (+) and (-) was also found (Figure 4.34). The response of selection for (+) is about two times greater than that of selection for (-).

The results of Figure 4.34 also show that the responses to selection for (+) and (-) were slightly higher than that for (H) and (L), respectively, but the difference was not significant.

Five kinds of selection in bidirectional selection were executed as follows:

(1) H-H: Individual plants with a high frequency of the loss were selected in the families derived from the parents with a high frequency of the loss event. The progeny of this kind of selection are presented in Figure 4.30: SA-1, SA-2, SA-5, SA-6 and in Figure 4.31: SB-1, SB-2, SB-5, SB-6.

(2) H-L: Individual plants with low frequency of the loss events were selected in the families derived from the parents with

high frequency of the loss event. Families of SA-3, SA-4, SA-7, SA-8 of Figure 4.30 and SB-3, SB-4, SB-7, SB-8 of Figure 4.31 belong to the progeny of this kind of selection.

(3) L-H: Individual plants with high loss frequency were selected in the families derived from the parents with a low loss frequency. This kind of selection yields the progeny as follows: SC-1, SC-2, SC-5, and SC-6 of Figure 4.32.

(4) L-L: Individual plants with low loss frequency were selected in the families derived from the parents with low loss frequency. Families of SC-3, SC-4, SC-7, SC-8 of Figure 4.32 are included in the progeny of this kind of selection.

(5) O: Individual plants without any loss were selected. SA-9 of Figure 4.30, SB-9 of Figure 4.31, SD-1 and SD-2 of Figure 4.33 are the progeny of this kind of selection.

The results of these kinds of bidirectional selection are shown in Figure 4.35. It indicates that:

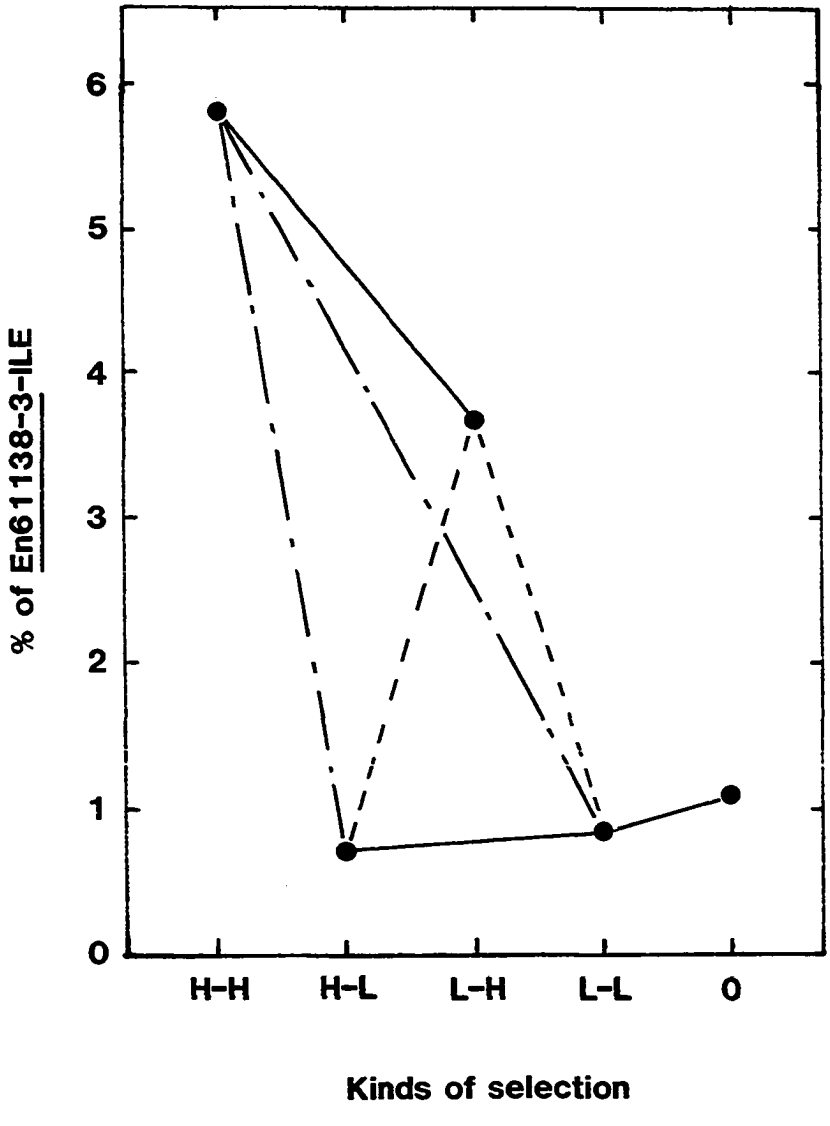
(1) The highest frequency of the loss events induced by En61138-3 was obtained in H-H compared with other classes of selection.

(2) Selection for L-L resulted in showing the lowest frequency of the loss event among all the classes of selection.

(3) The response to selection for H-H was greater than that for L-H, but the differences between them were not significant, indicating that the repetitive selection for the high loss frequency in the families derived from the parents with low loss frequency through three generations can reisolate the individuals with a high frequency of the loss event.

Figure 4.35. Responses to bidirectional selection on En61138-3-ILE in five classes

——— Shows no significant difference by t-test
—·— Shows significant difference at 5% level by t-test
- - - - - Shows highly significant difference at 1% level by t-test


6
5
4
3
2
1
0
% of En61138-3-ILE
H-H
H-L
L-H
L-L
0
Kinds of selection

(4) The responses to selection for H-L and L-L seemed to reach the same low level of the loss event frequency through three generations. Though the frequency of the loss events in H-L was slightly higher than that in L-L, the difference was not significant.

(5) The response to selection for H-H and L-L showed a highly significant difference between them.

(7) Highly significant difference in the response to reverse selection (H-L vs. L-H) was found, indicating that the selection for the individuals with high or low loss frequency can be made (also see Figure 4.34).

(8) The response to selection for "0" was not significantly different from that for L-L. This agrees with the results of the heritability of the "0" state of En61138-3 (Tables 4.35 to 4.38). Most of the offspring derived from the parents without any loss are in the "s-1" state of En61138-3 (section 4.3.2, Tables 4.35 to 4.38).

Another bidirectional selection was made on the kernels with (+) and without (-) loss sectors. Similar selection procedures were carried out as that in bidirectional selection for high loss frequency (H) and for low loss frequency (L) (Figure 4.35). Four kinds of selection were made in this selection for (+) and (-) kernels.

(1) (+) to (+): Kernels with loss sectors were selected from the parents which were derived from the kernels with loss sectors. SA-1 and SA-3 of Figure 4.30; SB-1 and SB-3 of Figure 4.31;

SC-1 and SC-3 of Figure 4.32 are the progeny of this kind of selection.

(2) (+) to (-): Kernels without loss sectors were selected from the parents which were derived from the kernels with loss sectors. SA-2 and SA-4 of Figure 4.30, SB-2 and SB-4 of Figure 4.31, SC-2 and SC-4 of Figure 4.32 are the progeny of this kind of selection.

(3) (-) to (+): Kernels with loss sectors were selected from the parents which were derived from the kernels without loss sectors. SA-5 and SA-7 of Figure 4.30, SB-5 and SB-7 of Figure 4.31, SC-5 and SC-7 of Figure 4.32 are the progeny of this kind of selection.

(4) (-) to (-): Kernels without loss sectors were selected from the parents which were derived from the kernels without loss sectors. This kind of selection includes the progeny such as SA-6 and SA-8 of Figure 4.30, SB-6 and SB-8 of Figure 4.31, and SC-6 and SC-8 of Figure 4.32.

Selections were carried out separately in the (H') class [Family (SA+SB)] and in the (L') class [Family SC]. The results are presented in Figure 4.36. It shows that:

(1) Selection for (+) to (+) yielded the highest loss frequency among all kinds of selections.

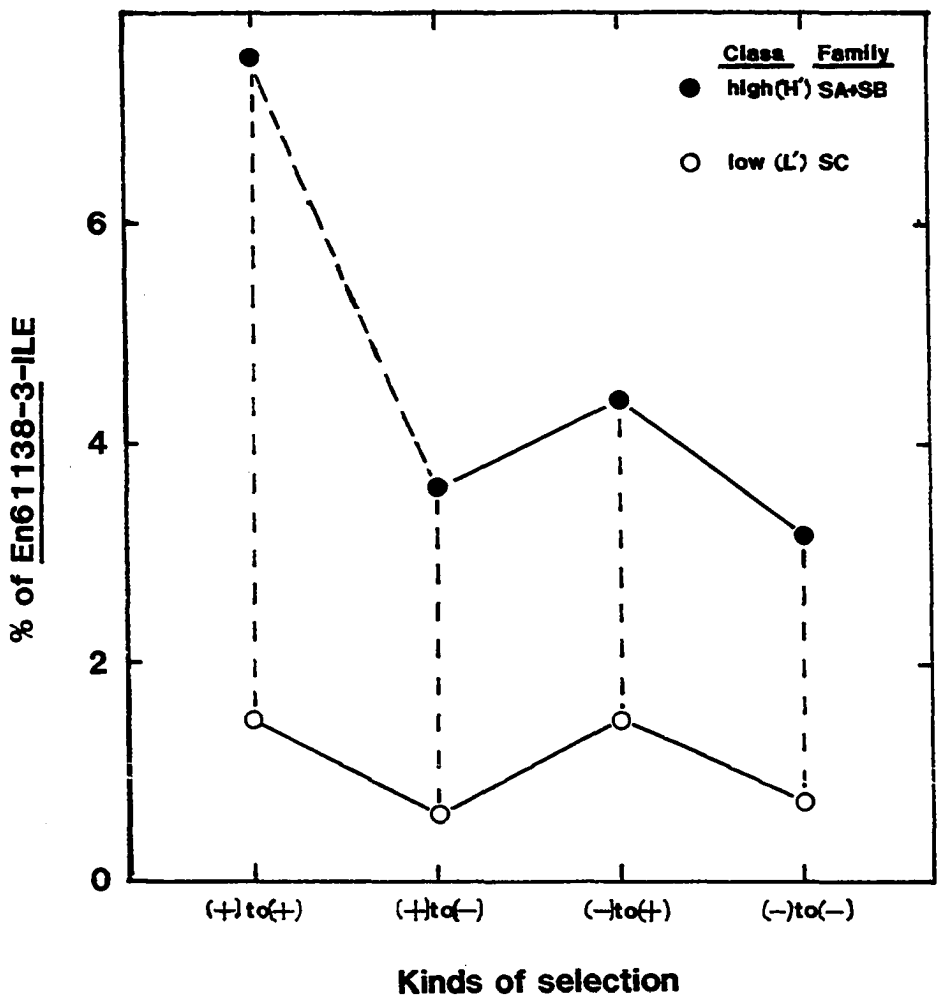
(2) Significant differences were found between (H') and (L') classes [Family (SA+SB) vs. Family SC] in each kind of selection. This difference is supposed to be caused by the number of modifiers.

Figure 4.36. Response to bidirectional selection on (+) and (-) kernels in families with high or low frequency of loss events in 1983

(+) Kernels with loss sectors were selected
(-) Kernels without loss sectors were selected

_____ Shows significant difference at 1% level by t-test between two selections

——— Shows nonsignificant difference by t-test between two selections


Class Family
high(H') SA+SB
low (L') SC
% of En61138-3-ILE
0
2
4
6
(+)to(+)
(+)to(−)
(−)to(+)
(−)to(−)
Kinds of selection

(3) The frequency of En61138-3-ILE was reduced significantly from (+) to (+) to (+) to (-) in (H) class, but no significant difference was found from (+) to (+) to (+) to (-) in (L') class.

(4) The changes of the responses to selection for (+) or (-) among the (+) to (-), (-) to (+), and (-) to (-) in (H') or (L') class were not significant, but nevertheless there still existed small responses to selections for (+) or (-).

From the results of (3) and (4), it is assumed that the significant difference between (+) to (+) and (+) to (-) selections in (H') class is due to some other factor(s) rather than the modifiers. The results of (3) and (4) also indicate that the effects of these factor(s) on changing (more likely increasing) the frequency of En61138-3-ILE are smaller than that of modifiers. The changes induced by the factor(s) on the frequency of En61138-3 will not be great in the absence of modifiers or in the situation in which these factor(s) are selected against or have been lost. The factor(s), in addition to the modifiers, probably can explain the significant response between the selection for (+) to (+) and (+) to (-) (Figure 4.36).

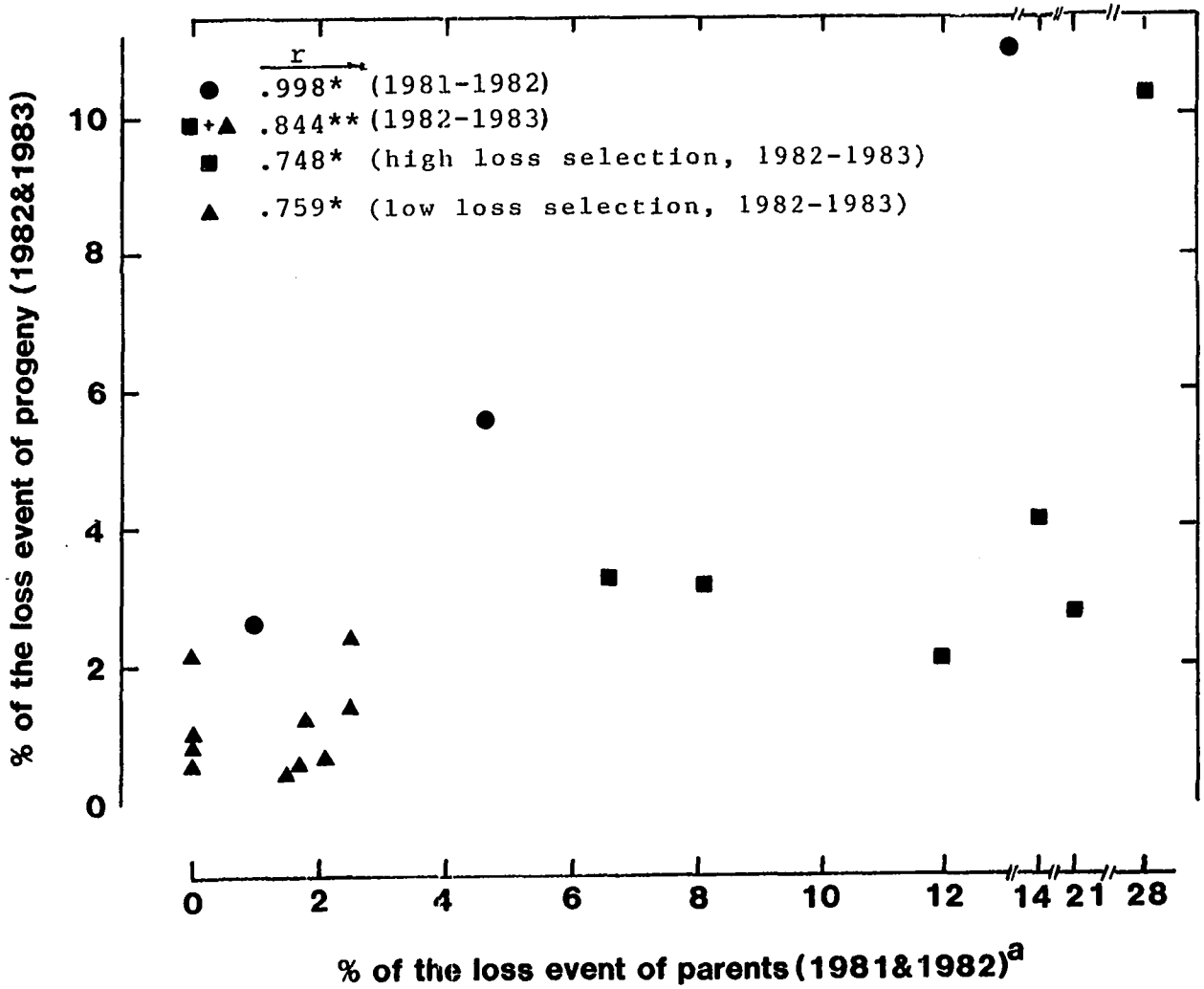
4.5.2.2. Heritability of En61138-3-ILE after selection

4.5.2.2.1. Parent-offspring correlation This test of relationship showed that a significant positive correlation (r=.9982* in 1982, r=.7463** in 1983) is found between the frequency of the loss event of the parents and that of their corresponding progeny after selection for the high and low loss frequency, respectively, in different families (Figure 4.37). This suggests that the selection can be made and the

Figure 4.37. Heritability of En61138-3-ILE after bidirectional and unidirectional selection.

---

[a]1981 parents are 81 0921/1032-8t (Table 4.13, line 18), 81 0902/1034-3 (Table 4.13, line 32) and a bulked sample (see footnote of Table 4.57, Family 3). 1982 parents with high loss frequency are the parents of SA-1+SA-2, SA-5+SA-6, SB-1+SB-2, SB-5+SB-6, SC-1+SC-2, SC-5+SC-6 (see Figures 4.30 to 4.32). 1982 parents with low loss frequency are the parents of SA-3+SA-4, SA-7+SA-8, SB-3+SB-4, SB-7+SB-8, SC-3+SC-4, SC-7+SC-8 (see Figures 4.30-4.32).


r
.998* (1981-1982)
.844** (1982-1983)
.748* (high loss selection, 1982-1983)
.759* (low loss selection, 1982-1983)
% of the loss event of progeny (1982&1983)
0
2
4
6
8
10
0
2
4
6
8
10
12
14
21
28
% of the loss event of parents (1981&1982)a

relative level of the En61138-3-ILE is heritable (also see section 4.5.2).

Because of the extremely high frequency of the loss event in parent 81 0911/1032-8t, it was not included in the correlation analysis for 1981-1982 data in order to avoid unexpected effects of this parent on the frequency of En61138-3-ILE.

4.5.2.2.2. Heritability of En61138-3-ILE after unidirectional selection Though the parent-offspring correlation is significant in bidirectional selection scheme, it is not clear that this relationship is due to selection for high loss or for low loss only. Therefore, it is advisable to determine the heritability of the frequency of the loss event in the unidirectional selection.

The parent-offspring correlations were estimated from the data of 1982-1983. The results shown in Figure 4.37 indicate that the heritability of the frequency of the loss event in each unidirectional selection is significantly high (r=.7482* for high loss selection, r=.7585* for low loss selection). This suggested that the heritability of the frequency of the loss event is high no matter what the direction of the selection.

In an attempt to determine the heritability of "zero" En61138-3-ILE, a contingency χ^2-test was used to estimate the transmission of the "zero" level of En61138-3-ILE to the progeny from the parents. The results obtained from the testcross progeny with their corresponding parents showed that only one comparison had a significant χ^2-value. Fourteen out of 36 comparisons kept the "zero" level of En61138-3-ILE in the progeny, 21 of these 36 comparisons expressing a low frequency of

En61138-3-ILE in the progeny were not significantly different from their corresponding parents (Table 4.59). Comparing with the results of Table 4.35 to 4.38, it is evident that the "zero" level of En61138-3-ILE belongs to the group of the "s-1" state (section 4.3.2, 4.5.2.1) of the En61138-3-ILE. The contingency χ^2-values show that the same potential to express the En61138-3-ILE can be transmitted to the progeny through the parents. The occurrence of mutation of the loss events from "zero" to "s-1" is probably only due to random change.

Table 4.59. Contingency χ^2-test on the frequency of En61138-3-ILE of the "zero" parents with their corresponding progeny

Crosses	Round spotted[a] No loss	Round spotted[a] Loss	% of loss	χ^2-values
A. Parent 82 1309/1110-1	48	0	0	
Progeny (1983 ♂ number)				
1254-1	122	0	0	∞
-2	118	1	0.8	$.2220^{ns}$[b]
-3	130	1	0.8	$.2754^{ns}$
-4	137	1	0.7	$.3074^{ns}$
-5	75	0	0	∞
-7	138	1	0.7	$.3120^{ns}$
-8	95	1	1.0	$.1259^{ns}$
-9	54	0	0	∞
-10	116	3	2.5	$.2175^{ns}$
-11	23	1	4.2	$.1268^{ns}$
-12	69	0	0	∞
B. Parent 82 1246/1118-4	154	0	0	
Progeny (1983 ♂ number)				
1255-4	91	2	2.2	1.1981^{ns}
-6	143	6	4.0	4.4221*
-7	95	2	2.1	1.1238^{ns}
-10	115	2	1.7	0.8319^{ns}
-12	57	0	0	∞
-1t	143	5	3.4	3.4188^{ns}
-5t	76	1	1.3	0.1255^{ns}

[a] The shrunken nonspotted colored kernels are not listed but appear approximately one-half in all the progeny.

[b] ns = Nonsignificant.

* Significant at .05.

Table 4.59. Continued

Crosses	Round spotted[a] No loss	Loss	% of loss	χ^2-values
C. Parent				
82 1256/1113-10	92	0	0	
Progeny (1983 ♂ number)				
1307-1	174	2	1.1	$.0778^{ns}$
-2	159	0	0	∞
-3	123	3	2.4	$.8132^{ns}$
-4	106	0	0	∞
-5	112	0	0	∞
-8	124	1	0.8	$.0238^{ns}$
-9	173	0	0	∞
-10	139	0	0	∞
D. Parent				
82 1252/1120-2	116	0	0	
Progeny (1983 ♂ number)				
1308-1	147	1	1.0	0.7868^{ns}
-3	127	4	3.1	1.9388^{ns}
-4	115	0	0	∞
-5	107	1	0.0	0.0013^{ns}
-8	156	1	0.6	0.0232^{ns}
-11	119	1	0.8	0.0003^{ns}
-7t	107	0	0	∞
-9t	112	2	1.8	0.5221^{ns}
-11t	41	0	0	∞

5. DISCUSSION

5.1. Genetic Characterizations of En61138-3-ILE

5.1.1. Genetic evidence

McClintock (1951) clearly identified the positions of breaks induced by Ac at Ds sites both genetically and cytologically. These breaks were observed at the pachytene stage in meiosis. McClintock (1951) further observed chromosomal duplications, deficiencies, inversions and translocations resulting from these breaks. Both the genetic and cytological evidence on the Ds-induced chromosome breakage were established in these early studies (McClintock, 1951, 1978).

The Ds-induced chromatid type of BFB cycle on chromosome 9 in maize continues in the mitosis of gametes and in the endosperm cells as chromosome replication and fusion proceed through each cell division. This type of BFB cycle results in variegation patterns of mutability on the kernels. But the chromatid type of BFB cycle ceases when a gamete containing a broken chromosome enters fertilization to form a zygote. The broken chromosome then heals and fusion would cease.

In this study, the genetic evidence is presented for a multiple gene loss of a, En, and Sh and, in addition, the inclusion of the Et gene on the long arm of chromosome 3. However, the cytological confirmation which would strongly support the loss events induced by En61138-3 is not available. It could be that the chromosome breaks induced by the En61138-3 allele might form the chromatid type of BFB cycle in microsprogenesis and in endosperm cells during the mitotic cell division.

By using the same procedure as McClintock (1951) did on Ds-induced chromosome aberrations, the cytological evidence for En61138-3-ILE might be obtained, but most of this loss event on the kernels occurs at a late stage during the endosperm development and is less frequent so that it would be difficult to rescue.

The En61138-3-ILE, including the A function, the En element, and the Sh gene on chromosome 3, is the first case of transposon-induced breaks among the maize transposable elements outside the Ac-Ds system. In the tests of the possibility of the Ac involvement as the major factor inducing losses at the a locus, it has been proved that an active Ac is not a component of the En61138-3 complex and, in addition, there is no active Ac elsewhere in the genome (Table 4.2). Therefore, it is evident that the chromosome breakage is not induced by Ds which responds to Ac, since no Ac is present. It can be concluded that the chromosome breakage involves only En.

The loss of the chromosome segment induced by En61138-3 could be detected on the seedling leaves of the crosses of a et/a et x En61138-3/a-m-1 sh (Cross 4.1.3G) (Figure 4.9, Table 4.10). But the frequency of the mutant seedlings with virescent stripes is much lower than that on kernels derived from the testcrosses (0.22% of the mutant seedlings compared with the average frequencies of En61138-3-ILE per ear: 7.8% of 1981, 6.7% of 1982, and 2.6% of 1983, see Figure 4.28). This difference is probably due to the different plant tissues (leaves vs. kernels) resulting in different frequencies of the occurrence of En61138-3-ILE.

A germinal loss of En61138-3 was not found in this study. One of the reasons is that the En61138-3 includes a deletion of a, En, and Sh,

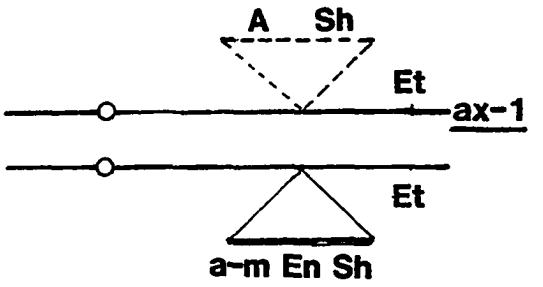
a similar deficiency as the ax-1 allele on chromosome 3. As a result, the transmission of the En61138-3 allele is expected to be reduced or even nonexistent through male gametes (ax-1 is transmitted with variably reduced frequency through male gametes, Natarajan (1981)). The other reason is that the En61138-3-ILE occurs at a very low frequency. Therefore, it might be difficult to detect the very low frequency of germinal loss induced by En61138-3 in male gametes.

In an attempt to determine the extent of the segment loss due to En61138-3, it is assumed that white sectors (stripes) would be expressed on green seedling leaves in the heterozygote ax-1/En61138-3 (Figure 5.1), because the ax-1 allele carries a small deletion including A, Sh and a chlorophyll controlling segment on chromosome 3. If the En61138-3-ILE occurs in the heterozygote ax-1/En61138-3, the deficiency region of ax-1 should be exposed resulting in white sectors (stripes) (so chlorophyll synthesis will appear on the green seedling leaves). Unfortunately, among 428 seedlings in the greenhouse seedling bench, no case was found of white sectors (stripes) on green leaves in the winter of 1982. These seedlings were derived from round spotted kernels with colorless shrunken sectors (therefore, evidence of the presence of the ax-1 allele) from crosses of ax-1/a-m-1 sh x En61138-3/a-m-1 sh (Cross 4.1.3A) and ax-1/a et x En61138-3/a-m-1 sh (Cross 4.1.3C).

There are several reasons for the failure of detecting the mutant seedlings with white sectors (stripes) on green leaves. One possibility is that the white sectors (stripes) caused by the exposure of the ax-1 allele might be due to the loss being a late-occurring event. Examination of leaves took place at the fourth and fifth seedling leaf stage on these

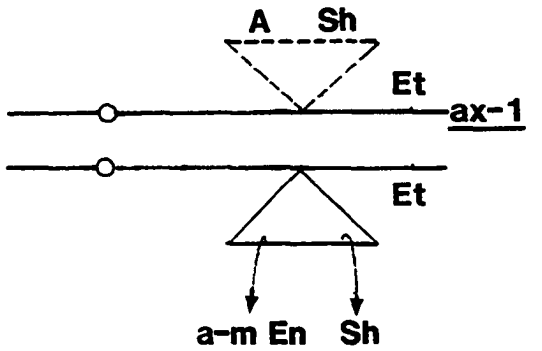
Figure 5.1. A diagram illustrating the loss of a-m En Sh from chromosome 3 results in the exposure of the deficiency of ax-1 on the leaves of the seedling leaves of the crosses of ax-1/a-m-1 sh or ax-1/a et and En61138-3/a-m-1 sh

A Sh
------- represent a deficiency of A Sh and a segment controlling chlorophyll synthesis on ax-1 chromosome



green leaves

Loss of a-m En Sh



white stripes
on green leaves

428 seedlings which were at the early stage of seedling development. Another possibility that white sectors (stripes) were not detected on the green leaves is that the gene controlling chlorophyll synthesis in the deficiency region of ax-1 is possibly proximal to the a En complex and is therefore not included on the segment that is lost from chromosome 3 induced by En61138-3.

5.1.2. Distribution of the loss events on kernels

The Poisson distribution of the frequencies of the En61138-3-ILE on kernels in six families of 1981 has been tested (section 4.1.3.6). The results show that the loss sectors are not randomly distributed on the kernels except in Family 6 (Table 4.12). The randomness was determined by χ^2-test (see section 3.7.4. for methods). This is because the observed values of the loss events occurring two or more than two times on the kernels are higher than their expected values (Table 5.1). The observed and expected values were estimated by the Poisson distribution formula (see section 3.7.4).

In an attempt to relate the frequency of En61138-3-ILE in male parents for each family (Cross 4.2.1A) and the results of tests for Poisson distribution of the loss sectors on kernels in each family, the trend was found as follows:

Family no.	1	2	3	4	5	6
Frequency of En61138-3-ILE of parents for each family (%)	14.1	28.2	10	9	7.2	1.7
χ^2-values in test for Poisson distribution	9483.8**	261.6**	27.1**	31.2**	79.2**	3.2^{ns}

Table 5.1. Observed and expected values of the kernels with different number of loss sectors of the crosses of a-m-1 sh/a-m-1 sh x En61138-3/a-m-1 sh (Cross 4.2.1A) in fitting the Poisson distribution[a]

1981 family[b]		Number of sectors on the kernels						
		0	1	2	3	4	5	>5
1	obs.	3227	127	26	9	11	3	13
	exp.	3089.66	310.23	15.58	0.52	0.01	0	0
2	obs.	5448	150	7	3	0	1	2
	exp.	5424.18	183.67	3.11	0.04	0	0	0
3	obs.	1579	50	7	0	1	0	0
	exp.	1570.39	65.23	1.35	0.02	0	0	0
4	obs.	2384	79	9	0	0	0	0
	exp.	2376.84	92.34	1.79	0.02	0	0	0
5	obs.	3527	108	13	1	0	0	0
	exp.	3514.54	131.95	2.48	0.03	0	0	0
6	obs.	3180	60	2	0	0	0	0
	exp.	3178.63	62.75	0.62	0	0	0	0
Combined	obs.	19345	573	64	13	12	4	15
	exp.	19147.84	858.62	19.25	0.29	0	0	0

[a]Expected values are calculated by the formula of Poisson distribution (see section 3.7.4).

[b]Source of each family: see footnote of Table 4.12.

which shows that a family derived from parent with a higher frequency of En61138-3-ILE shows that the distribution of the loss events in this family would have a greater degree of deviation from Poisson distribution. From these observations, it is evident that modifiers could increase the frequency of occurrence of loss events on kernels in addition to increasing the frequency of En61138-3-ILE on the ear.

The extremely high En61138-3-ILE value of 28.2% of one parent is probably due to other unknown reasons (e.g., environmental effects) rather than modifiers. Therefore, the χ^2-value was not greater than the parent with 14.1% of En61138-3-ILE. The χ^2-value of Family 5 is higher than that of Family 3 and Family 4 (79.4 vs. 27.1 and 31.2) (Table 4.12). The reason for this is that Family 5 is a highly heterogenous population with respect to the modifiers. A bulked sample with large loss sectors is equal to the parent of Family 5.

5.1.3. Transposition of the unique En in En61138-3 complex

In studies of the transposition of Ac, Mp, and En elements, each showed that they transposed preferentially to a number of possible sites and moved at a restricted distance on the same chromosome (Brink, 1958; McClintock, 1956a, c, 1962; Nowick and Peterson, 1981; Orton, 1966; Peterson, 1970). The movement of these elements is nonrandom with respect to the whole genome with a tendency to locate in distance close to the original sites.

Thus far, no other chromosome breakage has been identified as induced by En61138-3 allele except the breaks on the long arm of chromosome 3. Whether this En can move to other places on the same chromosome

or on other chromosomes and still retain the breakage ILE is still not known. Tests on this question are in progress.

5.2. Heritability of En61138-3-ILE on Family Basis

Parent-offspring correlation was used to judge the heritability of En61138-3 in several experiments in this study (Figures 4.11-4.15, 4.26-4.27, 4.37). The percentage of this correlation coefficient (rx100%) is identical to the standard unit heritability which was used to reduce the effect of genotype-environment interaction (Frey and Horner, 1957). Because the parents and their progeny were grown in two different generations (=years), such a correlation is suitable as a measure of the heritability of the frequency of En61138-3-ILE on a family basis in this study. Most of the results of the correlation analysis showed significantly higher heritability of the frequency of En61138-3-ILE. The range is about from 70% to 90% or higher (Figures 4.11-4.15, 4.26-4.27, 4.37). The reasons for some low or nonsignificant correlation (low heritability) and for inconsistent correlation values will be discussed in section 5.4.

5.3. States of En61138-3-ILE

The timing and frequency of the occurrence of the En61138-3-ILE on chromosome 3 cause the expression of variegated sectoring patterns. Different sizes and frequencies of the colored shrunken sectors were found on a-m-1 sh background in the testcross progeny (Figure 3.2, Table 4.1). These variegated sectoring patterns are designated as states of En61138-3 in this study (section 3.3.2).

Tests for the heritability of the states of En61138-3 by parent-offspring correlation show that the high heritability of the frequency of En61138-3-ILE mainly depends on the frequency of small-sized loss sectors (Figures 4.13 to 4.19). Most of the loss sectors occur at a later time during endosperm development. Parent-offspring analysis shows that the early-occurring loss events (large- and medium-sized loss sectors) are not heritable. The results obtained from the studies on the heritability of the states of En61138-3 on single kernel basis further support this conclusion (section 4.3.2). "s-1" state is a highly heritable trait (Tables 4.30, 4.31); "ℓ-1" and "zero" states are not heritable. Most of the progeny kernels with loss sectors derived from "ℓ-1" or "zero" state only show "s-1" state (Tables 4.32 to 4.38). Additionally, a negative correlation between the frequency of En61138-3-ILE and the size of the En61138-3-ILE was found (Figure 4.20). Early-occurring loss events have the lowest frequency among all loss events in a population, and late-occurring loss events show the highest frequency. Moreover, the result shows that the number of loss sectors are distributed randomly on the kernels without the effect of modifiers according to the Poisson distribution (Table 4.12). This indicates that the En61138-3-ILE occurring on kernels is a rare event compared with the kernels without loss sectors in a population.

From these observations, the possibility exists that the variegated patterns of the En61138-3-ILE (combinations of size and frequency of En61138-3-ILE) might also be a consequence of a Poisson distribution. This possibility could be tested by using the data of the testcross

progeny of 1983. Eleven classes were classified from "zero" loss event to "ℓ-5" loss event. Medium-sized loss sectors were merged with small-sized loss sectors. The same procedures were used to determine the expected values for each class by the probabilities of Poisson distribution (section 3.7.4). The results are presented in Table 5.2 and indicate that the variegated patterns of En61138-3-ILE have a similar trend as the distribution of the numbers of loss sectors on kernels (Table 4.12). Highly significant χ^2-values are due to the parents of these families with high frequency of En61138-3-ILE (=high frequency of modifiers). From these results, it is evident that the basic distribution of the variegated patterns of En61138-3-ILE is a Poisson distribution and therefore each pattern class occurs by chance alone. Because of this, the designated sectoring patterns of En61138-3-ILE in this study are not based on heritable features associated with different states of En61138-3 and it must be concluded that the sectoring patterns are the result of the random occurrences of En61138-3-ILE. Large-sized loss sectors should be with the lowest frequency (extremely rare loss event) compared with higher frequency of small-sized En61138-3-ILE.

The progeny population derived from the selected kernels with different sectoring patterns will still follow a Poisson distribution. This is the reason that most progeny kernels with loss sectors fall into the area of small-sized sectors irrespective of the parents with large-sized sectors or without sectors. As a result, the "ℓ-1" or "zero" state is not heritable with respect to the patterns of En61138-3-ILE yielded in the progeny. The Poisson distributions of En61138-3-ILE

Table 5.2. Observed values of the En61138-3-ILE of 16 families derived from a-m-1 sh/a-m-1 sh x a-m En Sh/a-m-1 sh (Cross 4.2.1C) in 1983 and the χ^2-values for test to fit Poisson distribution

Family[b] \ Events[a]	0	1	2	3	4	5	6	7	8	9	10	χ^2-value
1. SA-1 SA-2	1387	33	20	12	8	5	17	7	3	1	0	4283.44**
2. SA-3 SA-4	492	3	1	0	0	0	1	0	0	0	0	12.18^{ns}
3. SA-5 SA-6	3402	63	5	4	0	0	1	0	1	0	0	1243.23**
4. SA-7 SA-8	739	22	1	1	0	0	1	0	0	0	0	102.90**
5. SA-9	1077	8	0	0	0	0	1	0	0	0	0	2.6^{ns}
6. SB-1 SB-2	2378	50	9	2	3	5	3	1	1	0	0	7957.32**
7. SB-3 SB-4	864	2	0	0	0	0	0	0	0	0	0	0.02^{ns}
8. SB-5 SB-6	1857	31	7	3	5	5	1	1	1	0	0	13893.96**

[a] Event number 0-10 represents "zero," "s-1," "s-2," "s-3," "s-4," "s-5," "ℓ-1," "ℓ-2," "ℓ-3," "ℓ-4," and "ℓ-5" states, respectively (see Figure 3.2). Medium-sized sectors are merged with small sized sectors in this table.

[b] Different families and their sources are shown in Figures 4.30 to 4.33.

[c] ns = Nonsignificant.

*,** 5% and 1% significant levels, respectively.

Table 5.2. Continued

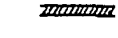
Family \ Events	0	1	2	3	4	5	6	7	8	9	10	χ^2-value
9. SB-7 SB-8	1311	9	1	0	0	0	1	1	0	0	0	12.00^{ns}
10. SB-9	720	18	0	0	0	0	0	0	0	0	0	0.23^{ns}
11. SC-1 SC-2	1562	40	3	0	0	0	2	0	0	0	0	8.57^{ns}
12. SC-3 SC-4	1151	8	2	0	0	0	0	0	0	0	0	62.50**
13. SC-5 SC-6	1655	42	4	5	0	0	1	0	0	0	1	872.49**
14. SC-7 SC-8	982	4	0	0	0	0	0	0	0	1	0	6.23^{ns}
15. SD-1	1112	4	1	0	0	0	1	0	0	0	0	19.01*
16. SD-2	1091	9	0	0	0	0	0	0	0	0	0	0.04^{ns}
Total	21510	346	54	27	16	15	30	10	6	2	1	71200.00**

of the parental population and of its progeny populations are shown in Figure 5.2 to illustrate these relations between parents and their progeny on the patterns of En61138-3.

It was previously noted that the "zero" and "s-1" states are due to a random change which is coincident with the Poisson distribution. But the average frequency of the En61138-3-ILE derived from "zero" state is lower than that from "s-1" or "ℓ-1" state (Tables 4.30 to 4.38). Furthermore, a significant difference in the frequency of En61138-3-ILE was found between the testcross progeny derived from with (+) and without (-) loss sectors (Figure 4.34, Tables 5.3 to 5.6). This indicates that the (+) kernels have the potential to give more kernels with chromosome breakage on ears than do the (-) kernels.

Moreover, bidirectional selection for (+) and (-) kernels from a single ear 80 2535/1857-6 was executed (Figure 4.29). This selection included two classes. One class with higher frequency of En61138-3-ILE (Families SA and SB, see Figures 4.30, 4.31) was designated as (H') class and the other class with lower frequency of En61138-3-ILE (Family SC, see Figure 4.32) was designated as (L') class. It was previously described that there was a significant difference in the response between selection for high (H) and low (L) loss frequencies (Figure 4.34). Similar results of this bidirectional selection for (+) and (-) show that each kind of selection [(+) to (+), (+) to (-), (-) to (+), and (-) to (-)] has a significantly higher frequency of En61138-3-ILE in (H') class than that in (L') class. This highly significant difference is due to the effects of modifiers (section 4.4.1.4). However, the

Figure 5.2. Poisson distributions of En61138-3-ILE in parental population (A) and in the progeny populations (B and C). Events 0-10 represent different patterns of En61138-3-ILE (see Table 5.2). Distribution (B) is derived from large-sized loss sectors of (A). Distribution (C) is derived from kernels without (-) kernels of (A).

 represents large-sized loss sector area

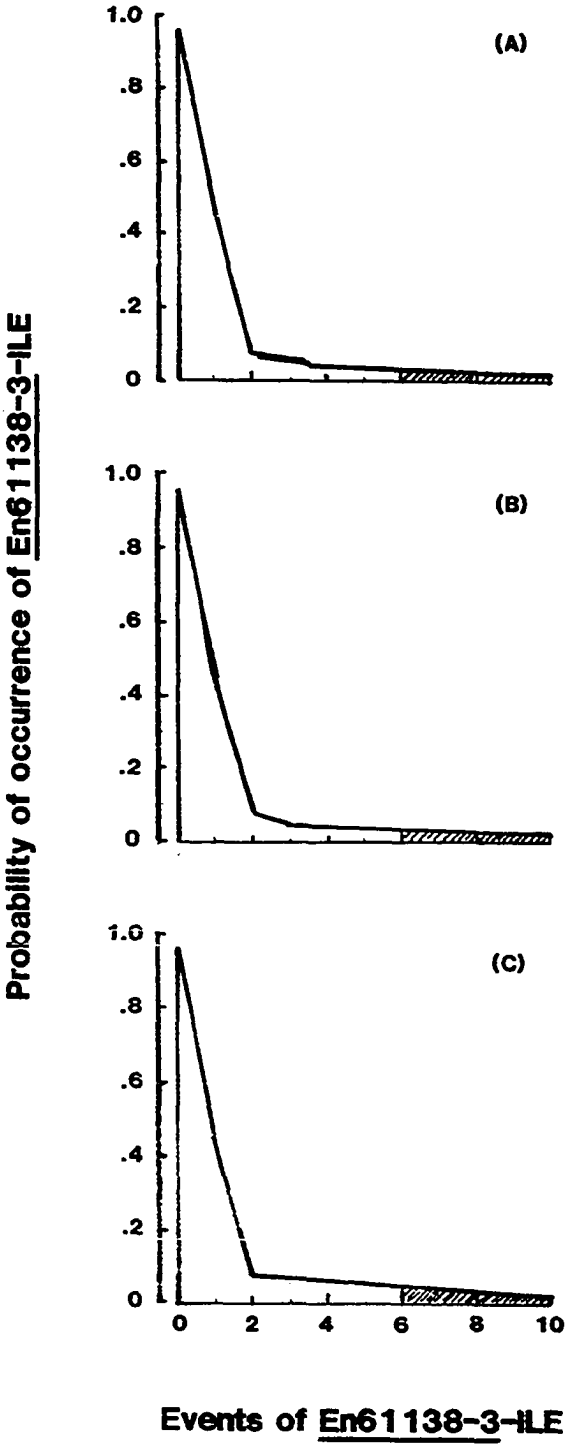

1.0
.8
.6
.4
.2
0
(A)
(B)
(C)
0
2
4
6
8
10
Probability of occurrence of En61138-3-ILE
Events of En61138-3-ILE

Table 5.3. Mean and standard error of percentage of sectors in six families from the crosses of colorless round spotted kernels with and without colored shrunken sector and a-m-1 sh/a-m-1 sh tester in 1981

Crosses \ Families[a]	1	2	3	4	5	6
With sector	8.6±2.3	3.3±0.9	5.3±1.7	3.9±0.8	3.5±0.6	1.9±0.5
Without sector	3.4±0.9	2.0±0.3	2.3±0.7	2.6±0.9	4.2±1.2	2.5±0.008
Mean ± S.E.	6.4±1.4	2.6±0.5	3.8±0.9	3.4±0.6	3.8±0.006	2.2±0.005

[a]Tables 4.13 to 4.18 are for the families 1, 2, 3, 4, 5, and 6, respectively.

Table 5.4. Analysis of variance for the occurrence of sectors in six families[a] from the crosses of colorless round spotted kernels with and without colored shrunken sector and a-m-1 sh/a-m-1 sh tester in 1981

Source of variation	df	MS
Families	5	0.0059[ns b]
Sector (families)	6	0.0053*
Error	168	0.0021

[a]See footnote of Table 5.3.

[b]ns = Nonsignificant.

*5% significant level.

Table 5.5. Mean and standard error of percentage of sectors in nine families[a] from the crosses of colorless round spotted kernels with and without colored shrunken sector and a-m-1 sh/a-m-1 sh tester in 1982

Crosses \ Families	1	2	3	4
With sector	6.25±0.91	8.59±1.36	6.11±1.29	2.64±0.38
Without sector	3.57±0.77	7.85±1.36	3.28±1.00	2.69±0.50
Mean ± S.E.	4.96±0.65	8.17±0.96	4.81±0.87	2.6710.33

[a]Families 1 to 9 are the families in Tables 4.19 to 4.27, respectively.

5	6	7	8	9
7.65±4.48	8.45±4.58	3.86±0.97	2.72±0.56	1.84±0.49
2.50±0.70	3.57±0.69	3.61±1.17	2.58±0.55	2.84±0.74
4.34±1.69	5.53±1.89	3.74±0.73	2.67±0.41	2.30±0.43

Table 5.6. Analysis of variance for the occurrence of sectors in nine families[a] from the crosses of colorless round spotted kernels with and without colored shrunken sector and a-m-1 sh/a-m-1 sh tester in 1982

Source of variation	df	MS
Families	8	999.9182
Sector (family)[b]	9	395.1896**
Error	9266	13.7649

[a]See footnote of Table 5.5.

[b]Sector represents the kernels with colored shrunken sectors vs. kernels without sectors.

**1% significant level.

frequency of En61138-3-ILE would not be changed greatly among the selections of (+) to (-), (-) to (+), and (-) to (-) in both (H') and (L') classes. Only a significant response was found from [(+) to (-)] to [(+) to (+)] in (H') class, and there was no significant difference between (+) to (+) and (+) to (-) in (L') class (Figure 4.36). It is interesting to find that the level of En61138-3-ILE could not be regained as much as that derived from the original (+) kernels by reverse selection. From these observations, it is evident that the changes between (+) and (-) kernels on the frequency of En61138-3-ILE are due to some other factors other than modifiers affecting the frequency of En61138-3-ILE in bidirectional selection for (+) or (-) kernels.

According to McClintock's (1949, 1950) reports, she found two states of Ds at the c-m-1 allele. State 1 of Ds shows a high frequency of acentric-dicentric formation. State II of Ds shows little or a low frequency of acentric-dicentric formation and is more stable genetically than State I. State I can mutate at a single mitosis to State II, but the converse is not true. The change from extreme State II to extreme State I requires several stepwise events that are reflected in the intermediate states. McClintock (1949, 1950) also indicated that repetitive selection through three or four generations on individual kernels showing breakage slightly higher than that in the parental strain was required to reisolate a derivative showing a high frequency of breakage from one that had mutated to the low frequency. These observations were interpreted according to McClintock as suggesting the existence of a number of active units within the Ds locus. The change from one

state to another involves a change in the number and/or distribution of these units within the Ds element.

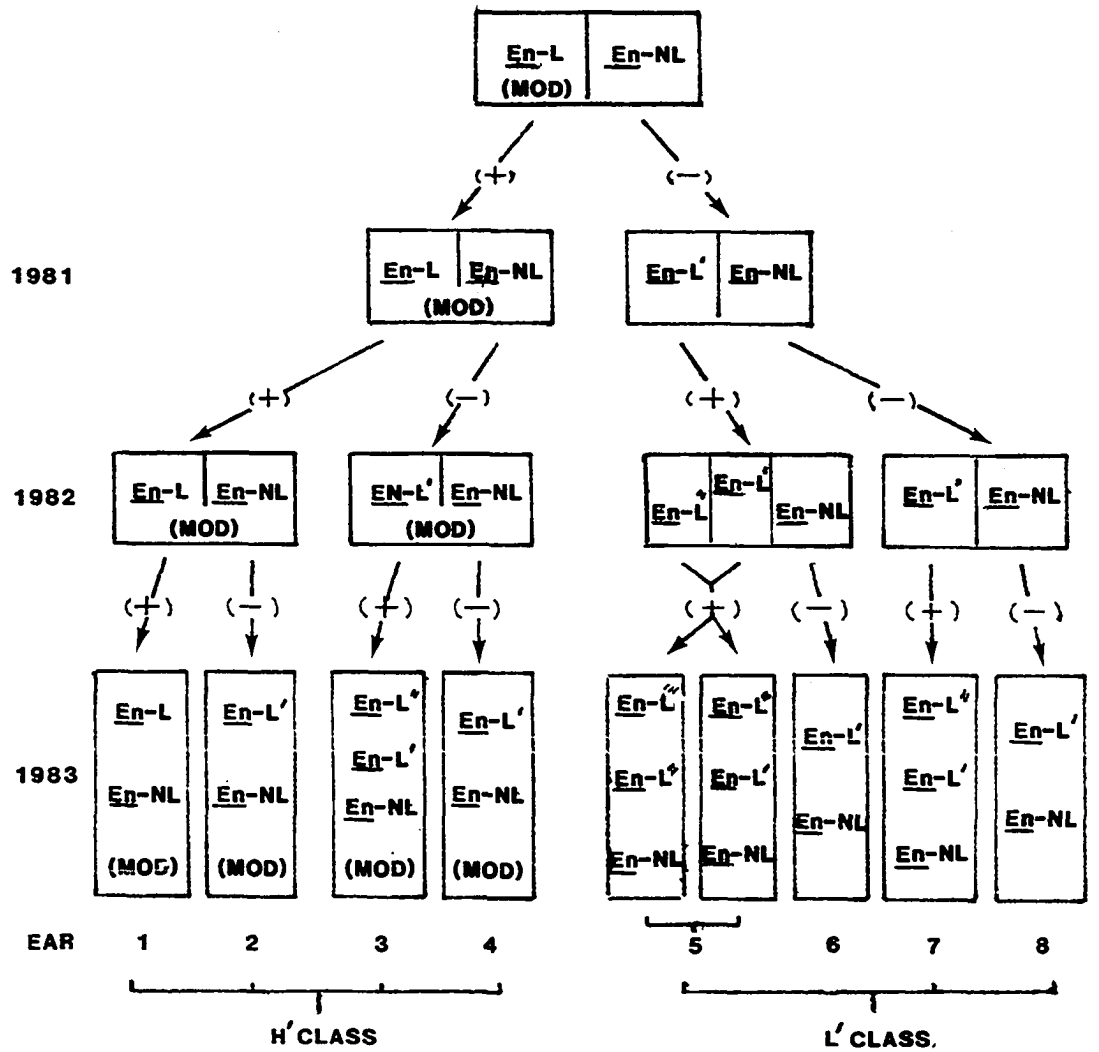
As a result, McClintock's (1949, 1950) hypothesis on the states of Ds might be applied to these unknown factor(s) participating in the selection for (+) and (-) kernels. Depending on the bidirectional selection scheme on (+) and (-) kernels (Figures 4.29 to 4.32), hypothetical changes of states of En of En61138-3 allele are presented in Figure 5.3. In Figure 5.3, it is assumed that En-loss (En-L) showing loss sectors on kernels (+) is a state of En that gives the highest frequency of En61138-3-ILE on the progeny ears. En-no loss (En-NL) showing no loss sectors on kernels (-) is another state of En that gives the lowest frequency of En61138-3-ILE on the progeny ears. Most of the En-L can revert to En-NL at a single mitosis during pollen development (pollen mitosis), but the En-NL cannot easily mutate to En-L at a single step. En-NL probably requires several steps to change its state to En-L. En-L' mutates from En-NL and gives a slightly higher frequency of En61138-3-ILE than that of En-NL. En-L" mutates from En-L' giving a slightly higher frequency of En61138-3-ILE than that of En-L' and En-L"' from En-L" and so on. En-L', En-L", and En-L"' are the intermediate states between En-L and En-NL. All of these intermediate states can easily revert to En-NL.

According to the hypothetical genotypes of En in each ear of 1983, shown in Figure 5.3, there is an indication that Ear 1 should express the highest frequency of En61138-3-ILE; Ears 2, 3, and 4 in (H') class should have a similar level of En61138-3-ILE, but Ear 3 will give a

Figure 5.3. A hypothetical change of states of En to illustrate the changes of the frequency of En61138-3-ILE for selection on (+) or (-) kernels

En-L = a state of En expressing loss sectors on kernels (+) gives the highest frequency of En61138-3-ILE
En-NL = a state of En expressing no loss sectors on kernels (-) gives the lowest frequency of En61138-3-ILE
En-L', En-L", En-L"' = intermediate states between En-L and En-NL states giving a slightly higher frequency of En61138-3-ILE from one that had mutate to the low frequency
(+) = kernels with loss sectors
(-) = kernels without loss sectors
MOD = modifiers

EAR 80 2535/1867-6



slightly higher frequency of En61138-3-ILE than Ears 2 and 4. Ears 5, 6, 7, and 8 in (L') class should show approximately an equal frequency of En61138-3-ILE, but Ear 5 will give the highest frequency of En61138-3-ILE among the four ears in (L') class and Ear 7 should be the second. Though the results of the bidirectional selection on (+) and (-) kernels are consistent with this hypothesis (see Figure 4.36), more repetitive selection from (-) to (+) through several generations would be required to confirm the changes of states of En representing the change of frequency of En61138-3-ILE in this study.

En-NL, En-L, En-L', En-L", and En-L"' states are probably due to the changes in the number of units and/or distribution of these units within the En element as McClintock hypothesized for the c-m-1 allele (McClintock, 1949, 1950). The (H') class, En-L state is seemingly complementary with modifiers to increase the frequency of En61138-3-ILE (7.5% of En61138-3-ILE in (+) to (+) selection in (H') class vs. 6.2% in (H)-(H) selection).

5.4. Factors Affecting the Rate of the Loss Event Induced by En61138-3

5.4.1. Modifiers

It is known that the frequency of En61138-3-ILE is determined by the modifiers rather than the En61138-3 allele in a population (section 4.4.1.4). The more modifiers, the greater the frequency of the En61138-3-ILE. In addition, these modifiers alter the patterns of En61138-3-ILE and change the mutation rates of En61138-3-ILE in different populations (Tables 4.11, 4.12, 5.1, 5.2).

In studies on the heritability of the En61138-3-ILE from parental main stalk and tiller to the progeny main stalk and tiller, there is a contradiction between the results of parent-offspring correlation and contingency χ^2-test (Figures 4.26, 4.27 vs. Tables 4.54, 4.55). This is because these two methods approach the estimate of the heritability of En61138-3-ILE in different directions. The correlation value represents the relationship between the average performance of the frequency of En61138-3-ILE of the progeny populations and that of their parents. The contingeny χ^2-values indicate whether the expression of En61138-3-ILE is at the same level as its parent on each progeny plant. But both of these two methods show that the heritability of the frequency of En61138-3-ILE is affected by modifiers. The total number of the modifiers in the progeny derived from the parents with a high frequency of En61138-3-ILE will still be high compared with that derived from the parents with a low frequency of En61138-3-ILE. As a result, the parent-offspring correlation shows a significant positive correlation value between the frequency of En61138-3-ILE of parents and that of their corresponding progeny. Since the individual progeny plants with variable frequency of En61138-3-ILE are derived from parents containing high frequency of modifiers (=high frequency of En61138-3-ILE), it is conceivable that the low percentage of nonsignificant χ^2-values should appear in this progeny population.

Some inconsistent results of the contingency χ^2-test are probably caused by the segregation of a high number of modifiers and the small sample size of the progeny population (Tables 4.54, 4.55).

It is known that the chromosome breaks of En61138-3-ILE are due to En. The excision of En from the a locus causes the loss including a, En, and Sh on chromosome 3. The process of excision of En in maize is unknown on molecular basis. However, the mechanisms of transpositions of transposons in prokaryotes such as Tn3, Tn10 have been extensively studied by several researchers (review, see Heffron, 1983; Kleckner, 1981, 1983).

Studies on the mechanisms of Tn3 transposition show that the short invert-repeated (IR) sequences (38 bp) at either end of the Tn3 transposon play a structural role for transposition. The IR probably function as sites at which the transposase can bind and carry out symmetrical enzymatic reactions with both ends of the transposon. The transposase encoded by tnpA gene on Tn3 is an essential enzyme for Tn3 transposition (Gill et al., 1979; Heffron et al., 1979).

Similar mechanisms for the transposition of Tn10 were found (review, Kleckner, 1983). Tn10 is a composite transposon with IS10 elements at its termini. The sequences of IS10 are closely associated with the transposition of Tn10. A particular symmetrical 6 bp sequence (GCTnAGC) located within a 9 bp target site (direct repeats) is the specific target site for insertion of Tn10 (Halling and Kleckner, 1982). This target DNA may be both recognized and cleaved by the IS10-encoded transposase. This transposase is responsible for both symmetrical recognition and cleavage of the target and symmetrical cleavage and joining of the transposon ends of the target.

A variety of chromosome rearrangements have been observed with transpositions of Tn3 and Tn10. The adjacent deletions were found in Tn3 and Tn10 (Nisen et al., 1977; Weinstock et al., 1979; Miller et al., 1980; Kleckner, 1981; Kleckner et al., 1979). These deletions extend from one terminus of the transposon to a variable site in the bacterial DNA. The element itself is not deleted. Internal deletions and inversions are specifically associated with Tn10. In some tetracycline-sensitive derivatives of a Tn10-containing bacteria, the deletions occur at the inside end of one IS10 element and extend across Tn10 into or beyond the tetracycline resistant gene and sometimes extends into adjacent chromosomal material (Kleckner et al., 1979; Ross et al., 1979). Furthermore, Kleckner et al. (1979) and Ross et al. (1979) found some tetracycline-sensitive derivatives isolated from Tn10-containing genomes that underwent the specific deletion of all Tn10 material between IS10 elements plus the inversion of a continguous DNA segment beginning at one IS10 inside and ending within that IS element or the adjacent chromosomal material.

All transposons are capable of excising precisely from this site of insertion. However, several types of excision events were found. For instance, three types of Tn10-associated excision events were established by Kleckner's group (see review, Kleckner, 1981, 1983). Precise excision (PE) is a deletion of Tn10 between two 9 bp-inverted repeats and exactly reconstructs the target site to its original wild type sequency. Nearly precise excision (NPE) is a deletion event similar in structure to PE but involving short repeat sequences that occur

within the Tn10 element, one near each end. Deletion of material between these repeats, analogous to excision between the 9 bp repeats in PE, results in excision of all but 50 bp of Tn10. Precise excision of the nearly precise excision remnant (NPE → PE) shows the 50 bp sequence remaining after NPE can be further excised to give complete restoration of the wild type target DNA sequence. These three events (PE, NPE, and NPE → PE) appear to be promoted by host-encoded DNA handling functions. Moreover, the alterations of direct and inverted repeat sequences such as small deletions and inversions would result in changing the frequency and precision of the excision events. All of these small rearrangements are presumed to occur as errors during DNA replication or repair process.

Altered RecBC protein in TexA$^-$ mutant strains of E. coli was found to increase the Tn10 PE. Several mutant genes in these Tex$^-$ mutants were identified for correction of base-pair mismatches. These results suggest that host-DNA handling functions are indeed involved in PE or NPE of Tn10 (Kleckner, 1983).

However, it should be noted that it is not clear as to what the relationship is between excision and transposition of the transposons. In some cases, the relationship exists and in others it does not (Starlinger, 1980).

Recently, molecular studies on Ds elements at Adh1 and Sh loci in maize show that the direct and inverted repeat sequences of the Ds element are probably responsible for the transposition of Ds element. Internal rearrangements of these sequences are supposed to be the basis for the "states" of Ds (Sachs et al., 1983; Döring et al., 1984b).

From all of these results on the transposons of prokaryotes and maize, it could be assumed that the process of excision of the En element might have a similar enzymatic interaction as that found in prokaryotes. Alterations of the direct or inverted repeat sequences probably result in changing the enzyme binding sites. It is probably a kind of transposase which is responsible for En excision. As a result, imprecise En excisions and errors in DNA replication or repair are presumed to occur to induce the deletion of a-m En Sh from chromosome 3. In addition, the adjacent deletions probably are induced by this En excision event leading to the loss of a, En, and Sh.

The interactions between the products of modifiers of the maize genome and the transposase are supposed to increase this imprecise excision of En element resulting in En61138-3-ILE. These interactions probably include the alterations of the structure or function of the transposase or alterations of the binding sites for transposase on inverted repeat sequence of En.

At the present time, the I element of En(Spm) controlling system has been isolated and sequenced at the Wx locus (Schwarz-Sommer et al., 1984), but more molecular studies on the structure and function of the En element are needed to resolve the questions of En excision in the En61138-3-ILE.

From all the results in this study on modifiers, it can be summarized that the properties of the modifiers are as follows:

(1) segregate independently,

(2) increase additively the frequence of the loss events on individual ears,

(3) show no dominant gene action,

(4) change the variegated patterns of En61138-3-ILE,

(5) exists in the testcross, selfing, and sibcross populations, and

(6) influence the mutation rate of En61138-3-ILE.

5.4.2. Effect of main stalk and tiller on En61138-3

Fowler and Peterson (1978) found that the En-v expression was quite different on the main stalk and tiller ear of the same plant. The kernels from the main stalk ear demonstrate a very reduced En-v expression with a low frequency of late-occurring mutations. The kernels from the tiller ear of the same plant show an active En-v expression with a pattern of mutability in higher frequency and earlier-occurring mutations. This increased level of mutability of the tiller ears is maintained when transmitted through the main stalk ear in the subsequent generation. These results indicate that heritable alterations of controlling elements can be produced by endogenous environmental factors present during normal plant development.

However, no significant differences on either the frequency of the loss event induced by En61138-3 or the size of the loss event between the main stalk and tiller of the same plant were found in this study (Table 4.51).

The comparisons of the exceptional pairs of main stalk and tiller of the same plant on the loss of mutability show that the altered level of the loss event could not be transmitted (Tables 4.48, 4.49).

Contrary to the conclusions of Fowler and Peterson (1978), the alterations of the loss of mutability on main stalk and tiller of the

same plant with En61138-3 probably are due to some external environmental factors and the number of modifiers, not the En controlling element.

5.4.3. Effect of the planting date on the frequency of En61138-3-ILE

Rhoades (1941) reported a negative relationship between the temperature and the frequency of Dt-induced mutations of the a-dt allele. Peterson (1958) compared the frequencies of mutations of pg-m → Pg and found a higher frequency of mutation at the higher temperature. Harrison and Fincham (1964) described the instability at the pal locus in Antirrhinum majus at different temperatures. The results showed that the higher temperature is accompanied by a lower frequency of mutation. pal-rec clones showed a higher frequency of spots at 15°C than that at 25°C. Heterozygotes pal-rec/pal-tub showed an extremely high frequency of somatic sectors at 15°C than that at 25°C.

In this study, no difference could be seen on the frequency of the En61138-3-ILE between two planting dates in three families (Tables 4.57, 4.58). The reason probably is the temperature range is not wide enough nor consistent (16 days apart; May 8 and May 24 in 1982) to affect the relatively low frequency of the En61138-3-ILE.

According to other results, though, the temperature may have strong effect; there is no general rule about its direction. It seems that different mutable systems respond differently to temperature.

5.5. Response to Selection on En61138-3-ILE as an Example to Illustrate a Possible Cause of Increasing Yield in a Plant Breeding Program

It has been discussed that the selection for the frequency of En61138-3-ILE can be made on either high or low loss frequency (sections 4.5.1, 4.5.2). These responses to the selections are significant (Figures 4.34, 4.35).

The results of selections on En61138-3-ILE imply that modifiers are selected for when selecting parents with a high frequency of En61138-3-ILE (sections 4.5.1, 4.5.2). The frequency of modifiers can be maintained through selfing or sibcross to the next generation. Therefore, selection for plants with a higher frequency of the En61138-3-ILE could be made and this could be maintained (section 4.4.1.4). However, it should be noted that selection for (+) to (+) in (H') class results in a significant response compared with other selections on (+) or (-) kernels (Figure 4.36). Selection for the kernels without (-) loss sectors from (H') class [(+) to (-)] will reduce the frequency of En61138-3-ILE but the response in the reverse selection [(-) to (+)] is not as high as [(+) to (+)].

Successful bidirectional selections on (H) and (L) are primarily due to direct or indirect accumulation of the number of modifiers through repetitive selection. But the limits of selection potential for a high frequency of the loss event is not known from the results of this study.

Maximum crop production is the most important goal of plant breeding programs. Yield is one of the factors dominating crop production. But

it is a complicated quantitative trait composed of several components, for instance,

Yield = number of ears per unit area x number of grain per ear x mean grain weight.

Each component contributes its effect to the outcome of yield. Therefore, selection for the components of yield separately or jointly rather than selection for yield per se is one of the plant breeding strategies. This approach is from studies with biometrical methods such as multivariate analysis, selection index, etc. The molecular basis for the yield and its components of the crops is not known.

In this study, it is known that modifiers could change the frequency of En61138-3-ILE on both individual ear and the progeny population through a trans-acting process. Responses to the selection on the frequency of En61138-3-ILE are significant in both directions (selection for high or low frequency of En61138-3-ILE) (Figure 4.35). This infers that the selections are affecting a modifier trans-acting process. It is supposed that the trans-acting process is an interaction of the products of the modifiers with the enzymes responsible for En excision event resulting in increasing chromosome breaks. Selection for components of yield or yield per se might imply that these selection procedures might involve similar trans-acting processes affecting some enzymes that are responsible for increasing yield.

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